

# Diversity of cotton-infecting gemini viruses

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## ABSTRACT

The whitefly-transmitted begomoviruses are an increasingly important group of pathogens globally. Two types of begomoviruses affect cotton and these occur in different geographic areas. The begomoviruses of the New World, such as Cotton Leaf Crumple Virus, are typical bipartite viruses. More recently the "Old World" cotton-infecting begomoviruses have been shown to be a novel new type of begomovirus complex. These consist of a monopartite begomovirus, which requires a satellite molecule (DNA  $\beta$ ) to infect cotton, as well as a non-essential satellite-like component (DNA1). The viruses, which cause cotton leaf curl disease on the Indian subcontinent, are examples of this recently identified group of pathogens. Comparisons of sequences indicates that these viruses have evolved in the areas in which they were found rather than being exotic, introduced pathogens.

## Introduction

Geminiviruses are single-stranded DNA viruses, which are transmitted plant-to-plant by insect vectors. The taxonomy of the Geminiviridae presently recognises four genera within this family of viruses. The genus *Mastrevirus* includes monopartite viruses with leafhopper vectors, which infect either monocotyledonous or dicotyledonous plants species. Viruses of the genus *Curtovirus* have monopartite genomes, leafhopper vectors and infect only dicotyledonous plants. The genus *Topocuvirus* encompasses only a single virus, *Tomato Pseudo-Curl Top Virus*, which has a monopartite genome, infects dicotyledonous plants and is the only virus known that is vectored by a treehopper. All the cotton-infecting geminiviruses identified to date fall in the genus *Begomovirus*, being transmitted exclusively by the whitefly *Bemisia tabaci*.

The vast majority of begomoviruses have genomes consisting of two approximately equally sized (~2750 nucleotide) single-stranded DNA circles (Figure 1). The first of these genomic components (DNA A) includes all of the virus-encoded products required for viral DNA replication, control of gene expression and encapsidation. DNA B encodes two products involved in virus movement in plants. A small number of begomoviruses have been identified which lack the DNA B component. For these viruses the homologue of the DNA A component is capable of inducing symptomatic infection of plants. The most prominent example of a monopartite begomovirus is *Tomato Yellow Leaf Curl Virus* (TYLCV; Czosnek & Laterrot, 1997). TYLCV is believed to have originated in the Middle East from where it has spread across the Mediterranean basin and, through the global trade in agricultural products,

has been introduced into the Caribbean and southern North America.

## Gemini virus-associated diseases of cotton

Diseases of cotton believed to be associated with begomoviruses have been reported from three regions of the world; the Americas, Africa and the Indian subcontinent. The New World diseases of cotton typically show leaf curling, distortion and varying degrees of yellow mosaic.

The "Old World" diseases of cotton typically exhibit leaf curling, vein swelling, vein darkening and the formation of enations on the veins on the undersides of leaves which frequently develop into leaf-like outgrowths (Figure 2). The best characterized of the Old World begomovirus-associated diseases of cotton is *Cotton Leaf Curl Virus* (CLCuV), a major constraint to cotton cultivation in Pakistan and western India (Briddon and Markham, 2000). Although the disease was noted in Pakistan in the late 1960s it was no more than a sporadic nuisance. However, in 1988 a block of a newly released cotton variety was affected in the vicinity of Multan and, by the late 1990s, all cotton growing areas of the country were affected. In addition, the problem spread eastwards into the cotton growing regions of India where the affected area continues to increase.

## New World cotton-infecting gemini viruses

The best characterized of the New World cotton-infecting begomoviruses is the agent which causes cotton leaf crumple disease in the southwestern United States. *Cotton Leaf Crumple Virus* is typical of the majority of begomoviruses in having a two component (bipartite) genome (Figure 1). Foliar symptoms of the disease are curling, crumpling and some mottling. In most years losses due to cotton leaf crumple disease are minor due to late infection of cotton crops. However, in years with high whitefly numbers the losses due to the combined effects of the whitefly and the virus it transmits can be significant.

A further disease of cotton, cotton yellow mosaic disease, has been reported from North and Central America. Only partial sequences of this virus are available. These indicate that the begomovirus associated with this disease is closely related to other malvaceous begomoviruses in this region.

## Old World cotton-infecting gemini viruses

The most thoroughly characterized of the Old World cotton-infecting begomoviruses is that associated with the *Cotton Leaf Curl Virus* (CLCuV) epidemic pres-

ently sweeping from Pakistan into western India. In the early 1990s this disease was shown to be associated with a begomovirus (Mansoor *et al.*, 1993). Extensive efforts to identify the presence of a DNA  $\beta$  component of the CLCuV-associated begomovirus were fruitless. However, clones of this virus were unable to induce typical disease symptoms in cotton, suggesting that either the virus was not the agent responsible for causing the disease or that some other component was required for induction of leaf curl symptoms (Briddon *et al.*, 2000). Subsequently two additional components (termed DNA 1 and DNA  $\beta$ ) were shown to be associated with leaf curl infected plants. Both components are circular, single-stranded molecules approximately 1350 nucleotides in length (Figure 3). DNA 1 is a satellite-like component that encodes a rolling-circle replication initiator protein, making it capable of self-replication in plant cells, but does not appear to play any essential role in the disease process (Mansoor *et al.*, 1999). DNA  $\beta$  in contrast is a symptom modulating satellite molecule that is essential for the induction of typical leaf curl symptoms by the associated begomovirus and requires this for *trans*-replication (Briddon *et al.*, 2001). Both DNA 1 and DNA  $\beta$  require the helper begomovirus for movement within and between plants by *trans*-encapsidation in the coat protein of the helper begomovirus.

Studies of the diversity of both the begomovirus and DNA  $\beta$  associated with the leaf curl epidemic on the Indian subcontinent have revealed a surprising finding. Although only a single DNA  $\beta$  "species" (now generally referred to as CLCuV DNA  $\beta$ ) has been identified, a number of closely related begomovirus species (including *Cotton Leaf Curl Multan Virus*, *Cotton Leaf Curl Kokhran Virus* and *Papaya Leaf Curl Virus*) have been shown to be able to induce leaf curl in cotton (in the presence of CLCuV DNA  $\beta$ ) and several more have been implicated (including *Cotton leaf Curl Alabad Virus* and *Cotton Leaf Curl Rajasthan Virus*; Mansoor *et al.*, 2003). It would appear, therefore, that leaf curl DNA  $\beta$  is capable of recruiting diverse begomovirus species. This has profound implications for the development of leaf curl resistant cotton varieties in that resistance is usually only against a single virus species. Conversely, this also suggests that a resistance strategy targeting DNA  $\beta$ , if this can be achieved, would be a more durable means of preventing losses due to leaf curl, since only one "species" of this molecule appears to be involved and it plays an essential role in the disease process.

The cotton-infecting begomoviruses occurring in Africa appear also to require a DNA  $\beta$  satellite. Idris and Brown (2000) have shown that leaf curl occurring in Sudan is associated with a monopartite begomovirus (*Cotton Leaf Curl Gezira Virus* [CLCuGV]) which is distinct from those occurring on the Indian subcontinent. Clones of CLCuGV have not been reported to be infectious to cotton, strongly suggesting that this virus also requires a satellite component to induce symptoms in

this host and a DNA  $\beta$  component has recently been shown to be associated with this virus (Idris and Brown, unpublished results). This is supported by the finding that begomoviruses of other malvaceous plants (including okra and hollyhock) in this region require a DNA  $\beta$  satellite (Briddon *et al.*, 2003).

## Relationships of cotton-infecting begomoviruses

Analysis of available sequences indicates that the cotton-associated begomoviruses have evolved independently in the geographical areas in which they are now found, rather than being recent, exotic introductions. Thus the begomoviruses causing leaf curl on the Indian subcontinent are distinct from those causing the same disease in Africa (Figure 4), although both require a DNA  $\beta$  component. Further evidence for this is provided by analyses of the sequences of DNA  $\beta$  components, which differ on the Indian subcontinent and Africa (Briddon *et al.*, 2003). These Old World, monopartite DNA  $\beta$  requiring begomoviruses, are in turn very distinct from the New World cotton virus *Cotton Leaf Crumple Virus*, a classical bipartite begomovirus.

## Conclusions

Begomoviruses are important pathogens of many crops in tropical, sub-tropical and, increasingly, more temperate regions. The begomovirus-DNA  $\beta$ -DNA 1 complexes in particular are a serious threat to cotton cultivation, showing a propensity to rapidly adapt to our changing agricultural landscape. Testament to this fact is the appearance in 2001 of a strain of the cotton leaf curl complex in Pakistan which is able to overcome the resistance to the virus which was introduced into local varieties by conventional breeding methods (Mansoor *et al.*, 2003).

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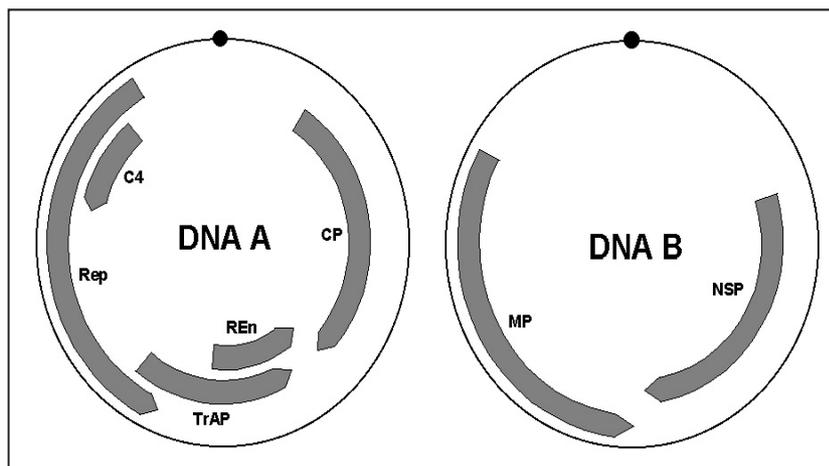
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**Figure 1.**

Typical genome arrangement of New World Begomoviruses. The position and orientation of genes are shown as arrows within the circles. Genes are labelled as coat protein (CP), replication-associated protein (Rep), transcriptional activator protein (TrAP), replication enhancer protein (REn), nuclear shuttle protein (NSP) and movement protein (MP). The function of C4 remains unclear. The black dot indicates the position of the origin of virion-strand DNA replication.



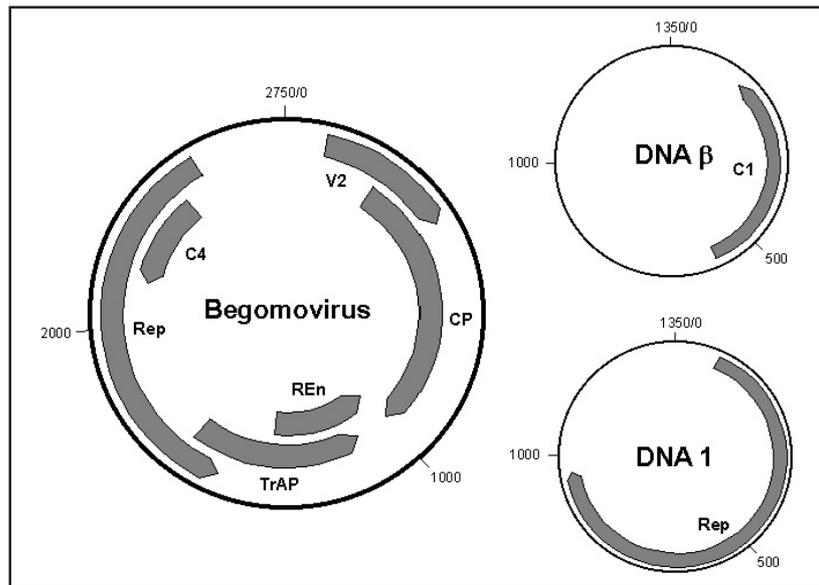
**Figure 2.**

Typical symptoms of Cotton Leaf Curl Virus in cotton showing swollen veins, enations and the leaf-like outgrowth on the mid-vein.



**Figure 3.**

Typical arrangement of genes on the components of begomovirus-DNA  $\beta$ -DNA 1 complexes. The positions and orientations of genes are shown as arrows. Genes are labelled as coat protein (CP), replication-associated protein (Rep), transcriptional activator protein (TrAP) and replication enhancer protein (REn). The function of the C4 gene of begomoviruses and the C1 gene of DNA  $\beta$  remains unclear. DNA 1 encodes a component-specific Rep.



**Figure 4.**

Phylogenetic dedrogram based on an alignment of the complete nucleotide sequences of the genomes (or DNA A genomic components) of selected begomoviruses. The viruses used were Cotton Leaf Curl Multan Virus (CLCuMV), CLCuMV isolated from okra (CLCuMV-Ok), Cotton Leaf Curl Alabad Virus (CLCuAV), Cotton Leaf Curl Kokhran Virus (CLCuKV), Cotton Leaf Curl Rajasthan Virus (CLCuR), Papaya Leaf Curl Virus (PaLCuV), PaLCuV isolated from cotton (PaLCuV-Co), Tomato Leaf Curl New Delhi Virus (TLCNDV), Ageratum Yellow Vein Virus (AYV), Indian Cassava Mosaic Virus (ICMV), African Cassava Mosaic Virus (ACMV), Tomato Yellow Leaf Curl Virus (TYLCV), Hollyhock Leaf Crumple Virus (HLCrV), Cotton Leaf Crumple Virus (CLCrV), Abutilon Mosaic Virus (AbMV) and Tomato Golden Mosaic Virus (TGMV). The tree was rooted on the sequence of Maize Streak Virus (MSV) a distantly related (to the begomoviruses) geminivirus. Vertical distances are arbitrary, horizontal distances are proportional to calculated mutation distances.

