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Cotton Leaf Curl Disease, an Emerging Whitefly Transmissible Begomovirus Complex

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ABSTRACT

In recent years leaf curl disease of cotton has become a major limiting factor in the production of cotton, which is an important fiber cash crop. In the last two decades or more, leaf curl disease in cotton took on epidemic proportions in Pakistan. In the early 1990's cotton leaf curl disease (CLCuD) appeared in the bordering parts of India in the states of Rajasthan and Punjab and spread to entire cotton growing areas in Rajasthan, Punjab and Haryana. The very early appearance of the disease devastated entire crops of susceptible varieties. This led to a ban on the cultivation of Barbadense cotton and the collapse of several popular cotton varieties. The characteristic symptoms of CLCuD are general stunting, upward or downward curling of leaves and thickening of veins which turn dark green. Enations of various shapes and sizes numbering up to twelve develop on the thickened veins on the abaxial side of leaves. Fewer, smaller balls are formed which sometimes fail to open. In the field CLCuD is a complex problem that needs an integrated management approach. Six species of the genus *Begomovirus*, family *Geminiviridae* viz. *Cotton leaf curl Alabad virus* (CLCuAV), *Cotton leaf curl Gezira virus* (CLCuGV), *Cotton leaf curl Kokhran virus* (CLCuKV), *Cotton leaf curl Multan virus* (CLCuMV), *Cotton leaf curl Rajasthan virus* (CLCuRV) and *Tomato leaf curl Bangalore* virus-Cotton [Fat] associated with DNA-β and DNA-1 (genus *Nanovirus*) are reported to induce CLCuD. All these begomoviruses are monopartite DNA-A, transmissible by the whitefly *Bemisia tabaci*. Begomoviruses are recognized as the most abundant and most severe viral pathogens of cotton, worldwide. Yield losses due to CLCuD amount to 60% or more if the disease appears at a very early stage of the crop. The aim of this review is to discuss and understand our current knowledge of CLCuD complex of cotton, and epidemiology, management and future prospects.

Keywords: *Bemisia tabaci*, CLCuD, DNA-β, epidemiology, integrated management, Nanovirus
Abbreviations: AYVV, *Ageratum yellow vein virus*; **BYVMV**, *Bhendi yellow vein mosaic virus*; **CLCuD**, Cotton leaf curl disease; **CMD**, *Cassava mosaic virus*; **ICTV**, International Committee on Taxonomy of Viruses; **NLS**, Nuclear localization signal; **PCR**, Polymerase chain reaction; **ToLCJAV**, *Tobacco leaf curl Java virus*; **TYLCCNV**, *Tomato yellow leaf curl China virus*; **TYLCV**, *Tomato yellow leaf curl virus*

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INTRODUCTION

Cotton is one of the most important fibre cash crops. In developing countries it is an important source of foreign exchange earning. *Gossypium hirsutum* is most widely grown and contributes about 80% of total cotton production. Cotton can be grown nearly throughout the year in India (ratoon), since climate conditions conducive to its growth are available in one or other part of the country. China is producing largest cultivated *Bt* cotton in nearly 6% of its cotton land area (3.3 million ha) (James 2005). India has the world's largest area devoted to cotton cultivation (about 9 million ha in 2005) (FAOSTAT 2006). Cotton leaf curl disease (CLCuD), earlier known as African leaf curl of cotton was first noticed in Nigeria on *G. peruvianum* and *G. vitifolia* (Farquharson 1912). Later a serious outbreak of this disease in Nigeria was described by Jones and Mason (1926). Subsequently this disease appeared in Sudan, Tanzania (Golding 1930; Kirkpatrick 1930; Prentice 1972), Pakistan (Hussain and Ali 1975) and India (Rishi and Chau-

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han 1994). A low incidence of CLCuV-K was also reported from the south of India in *G. barbadense* (Nateshan *et al*. 1996). The disease was first noticed in 1989 on few *G. bardadense* plants at an experimental farm in New Delhi. CLCuD came to prominence in 1993 when a few patches were affected in a block of a newly released variety of cotton (F-846) near Sriganganagar in Rajasthan. The disease continued to spread rapidly and the area affected was 500 ha in 1994-1995 that exceeded to 10,946 ha in 1996. In Punjab state, the disease was noticed in an area of 1500 ha during 1994. In 1997 there was outbreak of this disease in 80,000 ha in northern India. In the Haryana state, till 1996 disease was limited to about 20 ha only. During 1997-1998 seasons, there was sudden flare up of this disease in all the three states and as per estimates an area of about 219,610 ha was infested with CLCuD. The disease continued to spread and covered entire cotton growing areas of the northern India. During an early phase of the epidemic, the disease spread rapidly to the northern parts of India perhaps due to strong prevailing winds that carried the viruliferous whitefly. In Pakistan, this disease occurred in epidemic proportion in 1992-1993 and 1993-1994 affecting 889,000 ha and spread south into the Sindh region and across the border in north western India. The reduction in yield due to the incidence of CLCuD depends largely on the varieties grown, time of infection and severity of disease. During the 1980s CLCuD caused substantial losses to cotton crop in Pakistan (Ali *et al*. 1995) and later also reached India (Rishi and Chauhan 1994; Nateshan *et al*. 1996; Sharma *et al*. 2004b). The disease is transmitted by the vector, *Bemisia tabaci* Gennadius [Hemiptera; Aleyrodidae] in a persistent manner (Sharma and Rishi 2003).The importance of the disease stems from the fact that it is responsible for losses to the tune of 60%. Realizing the potential threats of CLCuD, it is feared that in future Indian cotton growing areas might be affected by this disease, as has happened in Pakistan. The disease was believed to be caused by cotton leaf curl geminivirus (Mansoor *et al*. 1993; Rishi and Chauhan 1994). Recent studies have shown that it is a complex disease with the involvement of six species of genus *Begomovirus* and satellite molecules, viz. DNA -1 (genus *Nanovirus*) and DNA-β (Rishi 2006). DNA -1, a circular single-stranded DNA of 1.4 kb associated with cotton leaf curl diseased cotton plants, reveals some sequence homology to genomic components of nanoviruses. *Geminiviridae* is the second largest family of plant viruses having geminate morphology and monopartite/bipartite ssDNA genome. Though geminiviruses are one of the earliest recorded virus diseases (Rishi 2006) during the last two to three decades these have emerged as devastating pathogens, particularly in the tropics and subtropics, causing huge economic losses and threatening crop production. Epidemics caused by re-emerging and newly emerging geminiviruses are becoming frequent even in regions, such as Uklana, Tohana, and Patra locations of Haryana state, that were earlier free from these viruses (Rishi 2004). Begomoviruses have emerged as more serious problems in a variety of crops e.g. cassava, cotton, grain legumes and vegetables. The major factors contributing towards the emergence and spread of new begomovirus diseases are the evolution of variants of the viruses, appearance of whitefly B biotype and increase in the vector population (Varma and Malathi 2003). The frequency with which new begomoviruses are appearing shows that these viruses are still evolving and pose a serious threat to sustainable agriculture, particularly in the tropics and subtropics (Brown *et al*. 1999, 2000). In recent years, *Tomato yellow leaf curl* virus (TYLCV) have also moved to temperate regions causing concern in the production of vegetables in greenhouses (Polston *et al*. 1999). Another concern is the emergence of diseases that are caused by a complex of begomovirus and DNA-β and DNA -1 (Briddon and Stanley 2005).

ECONOMIC SIGNIFICANCE

Economic losses induced by geminiviruses continued to pose a significant threat to world wide agricultural production particularly in the developing countries. The average cotton yield of Pakistan dropped by nearly 30%, resulting in losses of US $5 billion between 1992-1997 (Briddon and Markham 2000). Similarly, an annual loss of about $300 million is estimated on the basis of disease incidence in three crops viz. blackgram, munbean and soybean (Varma *et al*. 1992; Rishi and Sharma 2000). Cassava (*Manihot esculenta*) is the third most important food crop after cereals and grain legumes. It provides staple food to over 500 million people in tropical countries. Cassava mosaic disease (CMD) is a major constraint in cassava production, as it causes annual economic losses in the range of US $1300-2300 million in Africa alone (Thresh *et al*. 1998). CMD is another example of the emergence of a begomovirus disease caused by human intervention. Their widespread distribution and diversity, coupled with large-scale global movement of plant material and increased population of whitefly (Varma and Malathi 2003), introduction of biotypes in newer areas, suggest that these disease complexes pose a serious threat to global tropical and sub-tropical agro-ecosystems.

CLCuV: A COMPLEX OF VIRUS SPECIES

The development of molecular techniques has led to significant advances in the knowledge of geminiviruses, their genomes, and their role in causing leaf curl disease of cotton.

Etiology of the disease

Infected cotton plants exhibit curling of leaves which occur because of the uneven growth of veinal tissue and thickening of veins on the abaxial side (**Fig. 1**). The characteristic diagnostic symptoms are thickening and dark green colour of veins that is easily observed against sun light from a distance. Almost no yield is obtained from plants infected at an early stage, resulting in enormous losses to growers. Numerous other crops like okra, sunflower, tomato, chilli, tobacco, zinnia and weeds such as *Ageratum*, *Sida*, *Abutilon*, *Corchorus*, and *Achyranthus* (Mansoor *et al*. 1993; Zhou *et al*. 1998; Radhakrishnan *et al*. 2004; Sharma *et al*. 2004) also showed symptoms typical of those caused by whitefly-transmitted viruses. Confirmation of the association of whitefly-transmitted begomoviruses with many of these diseases could be obtained readily using serological (Nateshan *et al*. 1996; Harrison *et al*. 1997) and polymerase chain reaction (PCR)-based techniques (**Table 1**) (Harrison *et al*. 1997; Mansoor *et al*. 1998; Sharma *et al*. 2002). However, these reports do not provided conclusive evidence of etiology of these diseases. Recent work (Briddon *et al*. 2003, 2004) has revealed that there is association of Begomovirus, DNA-1 and DNA-β molecules.

Fig. 1 Infected cotton leaf on left side showing vein thickening, leaf curling and leaf-like enation which develop on the main vein on the reverse side of the symptomatic plant; right side is a healthy cotton leaf.

Table 1 Primers used for *Cotton leaf curl virus* detection

Sr. No.	Primer sequence	Reference
1.	Sense 5′-ATTATAAGCTTTCCGAGTGTTGTAGTTGAA CTGGATC-3′	Briddon *et al*. 2000
	Complementary 5′-ATTATAAGCTTTGGGACTGCACAA GTGTTTTCTAACC-3′	
2.	Forward 5′-ATAAAGTTTGAATTTTATTTC-3′	Radhakrishnan *et al*. 2004
	Reverse 5′-TAATATCAATTCGTTACAGAG-3′	
3	F 1800 5′-CCGG*GAGCTC*CCWC CTTTAATTTGAACBGG-3′	Nadeem *et al*. 1998
	R 1800 3′-GGWGGAAATTAAACTTGVCC*AGATCT*AACG-5′	
4.	CPF 5′-AATTATGTCGAAGCGAGCTGC-3′	Sharma *et al*. 2005b
	CPR 5′-TAATATCAATTCGTTACAGAG-3′	

Degenerate nucleotides are denoted as: Y=C, T; R=G, A; W=T, A; B=C, G, T; V=A, C, G.

Involvement of Begomovirus

The recent development of molecular techniques has led to significant advances in the knowledge of geminiviruses, their genome and role in causing leaf curl diseases of cotton. *Geminiviridae* family divides Geminiviruses into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*, depending on their vector, host and genomic characteristics (van Regenmortel *et al*. 2000; Fauquet and Stanley 2005). They have geminate (twinned) particles approximately 18-20 nm in diameter and 30 nm long, consisting of two incomplete T = 1 icosahedra joined together in a structure with 22 pentameric capsomers and 110 identical protein subunits. There are now 133 geminivirus species recognized by the International Committee on Taxonomy of Viruses (ICTV) of which 117 belong to the genus *Begomovirus* (Stanley *et al*. 2005), and there are almost 400 complete nucleotide sequences deposited in databases (Fauquet and Stanley 2005), reflecting their economic importance, enormous diversity, widespread geographic distribution and host adaptation. Complete nucleotide sequences of cotton leaf curl virus including strains are mentioned in **Table 2**. Recently, the Geminiviridae Study group (http: //www.danforthcenter.org/iltab/Geminiviridae/about.htm) proposed new species demarcation criteria, the most important of which being an 89% identity threshold between the complete DNA-A component nucleotide sequences of begomovieuses. Henceforth, CLCuV isolates are currently classified into several viral species based on the criteria accepted by ICTV for the genus *Begomovirus*. Geminivirus components vary in size between 2500 and 3100 nucleotides; each encodes two or more genes that are distributed between both the virion-sense and complementary sense DNA strands and are transcribed bidirectionally from an intergenic region which also contain origin of replication (Hanley-Bowdion *et al*. 1999). The majority of begomoviruses have bipartite genomes but an increasing number are being identified that have only a single component DNA-A. TYLCV is the most notable and economically most significant example of monopartite begomovirus (Navot *et al*. 1991). Transmission by whitefly, association of geminate particles with diseased plants, hybridization with a CLCuV DNA-A probe, cross reaction with monoclonal antibodies and PCR amplification with begomovirus DNA-A specific primers (**Table 1**) (Mansoor *et al*. 1993; Sharma 2002; Radhkrishnan *et al*. 2004) showed that CLCuD is associated with a begomovirus that is now known as *Cotton leaf curl Begomovirus* (CLCuV). Various isolates of CLCuV in India, Pakistan and the Sudan have only single DNA component resembling DNA-A of the Old World begomoviruses (Nadeem *et al*. 1997; Zhou *et al*. 1998; Idris and Brown 2002; Krithi *et al*. 2004). However, they show considerable diversity in their nucleotide sequences as shown in the phylogenetic tree (**Fig. 2**) of the CLCuV isolates characterized (as per ICTV standards) to date where sequence information is available for the complete genome. These isolates are grouped in five different species of *Begomovirus* based on nucleotide sequence identity, three of these species occur in Pakistan, and one each in India and the Sudan. The sequence identity of DNA-A components of the viruses causing CLCuD in Pakistan differ by 8-29% indicating variability amongst the viruses associated with the CLCuD epidemic. Maximum variability is in ORF AC4 whereas the CP is most highly conserved (Zhou *et al*. 1998; Krithi *et al*. 2004). The *Cotton leaf curl Gezira virus* (CLCuGV) isolate from the Sudan, where CLCuD has been endemic for over 70 years, is even more distinct as it shares only 74% sequence identity with one of the viruses from Pakistan (Idris and Brown 2002). Variability in geminiviruses has arisen through mutations, recombination and pseudo recombination (Varma and Malathi 2003).

The findings of Briddon *et al*. (2000) reported that

Table 2 Cotton leaf curl virus (CLCuV) isolates and sequences available in Genbank databases. #

Species	Geographical origin (strains names)	Genbank
Cotton leaf curl Alabad virus	Cotton leaf curl Alabad virus (CLCuAV-[802a])	AJ002455
(cotton leaf curl virus-Pakistan 3)	Cotton leaf curl Alabad virus (CLCuAV-[804a])	AJ002452
Cotton leaf curl Gezira virus	*Cotton leaf curl Gezira virus (CLCuGV)	AF155064
(Okra enation virus)	Cotton leaf curl Gezira virus – [Cotton] (CLCuGV-[Co])	AF260241
	Cotton leaf curl Gezira virus –(CLCuGV-[Hl:Cai])	AJ542539
[Hollyhock Cairo]	Cotton leaf curl Gezira virus – [Okra Egypt] (CLCuGV- [Ok:EG])	AY036010
	Cotton leaf curl Gezira virus – [Okra Gezira] (CLCuGV-[Ok:Gez])	AY036006
	Cotton leaf curl Gezira virus – [Okra Shambat] (CLCuGV-[Ok:Sha])	AY036008
	Cotton leaf curl Gezira virus – [Sida] (CLCuGV-[Si])	AY036007
Cotton leaf curl Kokhran virus	Cotton leaf curl Kokhran virus – (CLCuKV-[72b]	AJ002448
(Cotton leaf curl virus – Pakistan2)	Cotton leaf curl Kokhran virus – (CLCuKV-[806b])	AJ002449
	Cotton leaf curl Kokhran virus – [Dabawali] (CLCuKV-[Dab])	AY456683
	Cotton leaf curl Kokhran virus – [Faisalabad1] (CLCuKV-[Fai1]	AJ496286
Cotton leaf curl Multan virus	Cotton leaf curl Multan virus – (CLCuMV-[26])	AJ002458
(Cotton leaf curl virus – Pakistan1)	Cotton leaf curl Multan virus – (CLCuMV-[62])	AJ002447
	Cotton leaf curl Multan virus – [Faisalabad1] (CLCuMV-[Fai1])	X98995
	Cotton leaf curl Multan virus – [Faisalabad2] (CLCuMV-[Fai2])	AJ496287
	Cotton leaf curl Multan virus – [Faisalabad3] (CLCuMV-[Fai3])	AJ132430
	Cotton leaf curl Multan virus – [Multan] (CLCuMV-[Mul])	AJ496461
	Cotton leaf curl Multan virus – [Okra] (CLCuMV-[Ok])	AJ002459
Cotton leaf curl Rajasthan virus	*Cotton leaf curl Rajasthan virus – (CLCuRV)	AF363011

CLCuV species are those officially recognized by ICTV (Fauquet and Stanley 2005). * tentative species/strain name

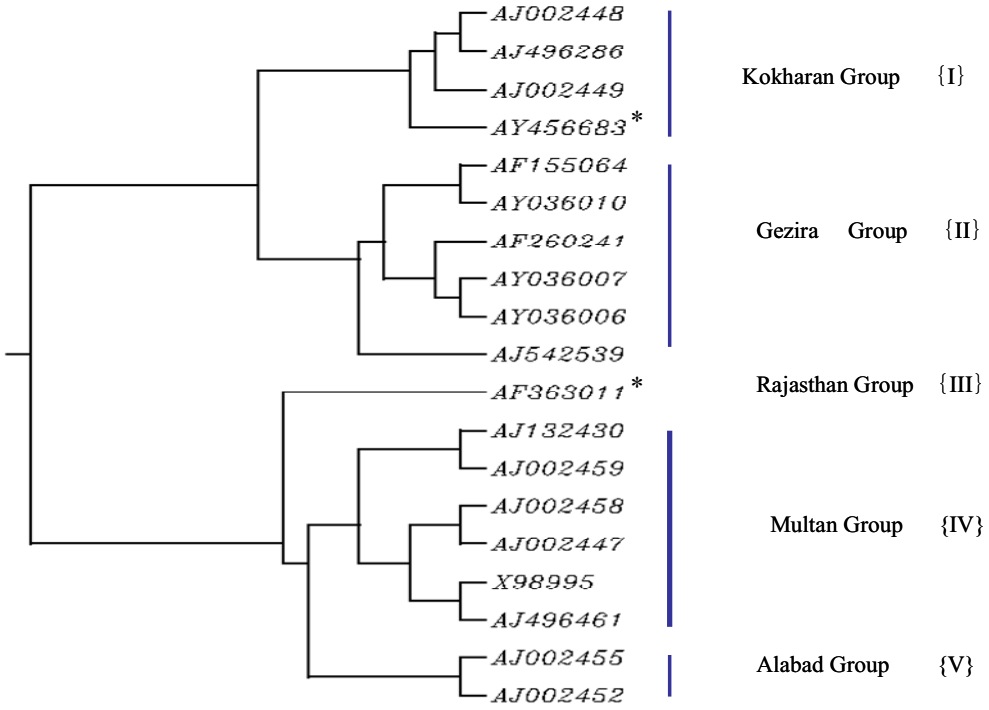

Fig. 1 Phylogenetic tree showing the relationship between isolates of cotton leaf curl virus (CLCuV) based on the multiple alignment of nucleotide DNA-A sequences using ClustalW (Thompson *et al.* 1994).
* Viruses reported in India.

plants infected with the cloned component of CLCuV DNA-A showed very mild symptoms and no vein thickening, and enations on the undersides of leaves. This was conclusive evidence that CLCuV DNA-A is not the only causal agent of leaf curl disease of cotton.

Involvement of subviral agents

Presently, it has been shown that in addition to Begomovirus there is involvement of two additional satellite molecules, namely DNA-1 and DNA-β. These satellite molecules depend on a helper virus for their replication but lack extensive nucleotide sequence homology to the helper virus (Mayo *et al*. 2005).

Satellite like DNA-1 molecule

A second major development occurred when another novel circular ssDNA component of approximately half the size of the begomovirus component was isolated from infected cotton plants (Mansoor *et al*. 1999). CLCuV DNA-1 is unrelated to geminivirus DNA but shows a close relationship to some genomic components of the *Nanoviridae*, specifically those that encode the Rep protein. It has the capacity to encode a 36.6 kDa Rep protein that is highly homologous to counterparts encoded by nanoviruses. DNA-1 has a typical stem-loop sequence containing the nanonucleotide TAATATTAC, which differs slightly from the highly conserved begomovirus motif but occurs in many nanovirus components (Briddon *et al*. 2004). However, DNA-1 is significantly larger than nanovirus components that are typically 1000-1375 nucleotides in length. The DNA-1 intergenic region, located between the 3′ end of the *Rep* coding region and the stem-loop, contains a particularly A-rich sequence. Homologues associated with *Ageratum yellow vein virus* (AYVV) and CLCuD referred to as nanovirus-like components (Briddon and Stanley 2005) are more closely related to some of these components than to the master Rep components. It is likely that the DNA-1 homologues originated from nanovirus components that became associated with begomoviruses during mixed infections and, as a consequence, changed from being aphid- to whitefly-transmitted. This necessitated a size increase from that of a typical nanovirus component to approximately half that of a begomovirus component. CLCuD DNA-1 is approximately half the size of helper begomovirus and encodes Rep. It can replicate autonomously although it depends on the helper begomovirus for encapsidation and movement both within and between plants. It is clearly related to nanovirus Rep-encoding components and most closely related to DNA-Rd components (Briddon *et al*. 2004). The DNA-1 is unrelated to other begomovirus DNAs and to the satellite DNA associated with ToLCV (Dry *et al*. 1997), which is significantly smaller (682 nucleotides) and depends on the begomovirus for its replication. However, it shows a close relationship to some genomic components of members of the *Nanoviridae*, specifically those that encode the Rep protein. Comparison of DNA-1 and DNA-Rd components suggests the increase in size of the nanovirus component may have occurred by inclusion of an A-rich region, possibly generated by a template slipping mechanism during replication. Despite sharing no significant sequence homology with their helper begomoviruses and dependence on the begomovirus for their maintenance, DNA-1 components could not strictly be defined as satellite DNAs according to recent ICTV guidelines because they replicate autonomously (Mayo *et al*. 2005). In view of their clear evolutionary origin, they could be considered simply as nanovirus-like. However, the fact that they play no essential role in proliferation of the helper begomovirus yet appear to be frequently associated with begomovirus diseases (Briddon *et al*. 2004) suggests that a new category of satellite component is necessary. Although the discovery of CLCuD DNA-1 did not resolve the etiology of cotton leaf curl disease, it represents another important step in the search for an additional component. Therefore, it is concluded that nanovirus-like DNA-1 component associated with infected cotton has no significant role in the disease and represents a satellite-like DNA.

Satellite DNA-β molecule

The involvement of a DNA satellite, DNA-β was found associated with some monopartite begomoviruses like CLCuV, AYVV, ToLCJV, BYVV infecting cotton, ageratum, tomato and okra have a symptom-modulating role in some host plants (Saunders *et al*. 2000; Briddon *et al*. 2003; Jose and Usha 2003; Radhakrishnan *et al*. 2004; Kon *et al*. 2006). With the discovery of DNA-β the dilemma of an infectious clone has been solved. It is therefore rightly named DNA-β because it functionally resembles the DNA-B component of bipartite begomoviruses. Transmission of both components and propagation of disease in cotton using whitefly transmission confirmed the etiology of disease as

similar to a DNA-β homologue isolated from ageratum used to show that AYVD caused by a monopartite begomovirus and DNA-β complex (Saunders *et al*. 2000). The motif, which DNA-β shares with geminiviruses, forms the loop of a predicted stem loop structure, which for geminiviruses, contains the nick site for initiation of virion-sense DNA replication. This sequence differs from that of DNA-1 and the majority of nanovirus components, from which DNA-1 is proposed to have evolved (Mansoor *et al*. 1999) to have a TAATATTAC loop sequence. The sequences of CLCuV DNA-β contain an A-rich region between nucleotides 766 and 984 and show 96% overall nucleotide sequence similarity with the majority of the nucleotide changes occurring just downstream of the nanonucleotide motif in a putative non-coding region. The function of this A-rich region is presently unknown but it can be deleted without effects on replication, encapsidation or symptom development (Tao *et al*. 2004). The role of this conserved sequence may be as a special type of "filler" sequence that can be used to maintain genome size. DNA-β requires the helper begomovirus for replication, movement in plants and insect transmission, presumably by *trans*-encapsidation in the begomovirus coat protein and alters the symptoms induced in some host plants (Briddon *et al*. 2003).

Recently two novel DNAs (referred to as SatDNA-II and SatDNA-III) isolated from cassava infected with bipartite begomoviruses in Tanzania have been reported (Briddon and Stanley 2005). These are GC-rich having approximately 1000 and 12000 nucleotides in length, quite distinct from all geminiviruses and subviral components. These satellites components need more detailed analysis as CMD is more pandemic affecting many central and east African countries (Legg and Fauquet 2004).

CLCuV HOST RANGE AND VIRUS HOST INTERACTION

The experimental host range of CLCuD using viruliferous *B*. *tabaci* includes cotton, tobacco, tomato, China rose, ageratum, okra, French bean and hollyhock (Sharma and Rishi 2003). It was interesting to observe that the disease incidence was often nearer downwind orchards that possibly brought the viruliferous whitefly (Rishi and Sharma, unpublished results). No disease appears on the desi cotton, *G*. *arboreum*; probably the initial source of infection may be weeds or the surviving infected ratoon cotton from the previous season. Some of the popular cotton varieties used in north-west India have played a major role in the epidemic. Varieties like F-846, RST-9, HS-2 HS-6 are more susceptible to CLCuD (Sharma *et*. *al*. 2005a). It appears that CLCuD problem could be managed most effectively by evolving and introducing resistant/tolerant varieties.

DNA-β might also play an important role in determining the host range of its associated begomovirus. For example, the bipartite begomovirus *Sri Lanka cassava mosaic virus* (SLCMV) normally infects cassava but is unable to infect ageratum. However, in the presence of DNA-β from ageratum, SLCMV can dispense with its DNA-B component and behave essentially as a monopartite begomovirus, infecting ageratum and producing a yellow vein phenotype. Thus, the association of begomoviruses with DNA-β components could provide greater opportunities for host-range adaptation and diversification. Despite having a profound effect on symptom development little is known about the function of βC1 i.e. its involvement in movement. Nuclear localization of the protein (Cui *et al*. 2005) indicates no direct role of βC1 in facilitating virus movement across the plasma membrane. The host response to βC1 expression suggests that it may be involved in reprogramming the infected cell to provide conditions more suitable for begomovirus replication. Alternatively, as gene silencing suppressors frequently induce pathogenic effect in plants (Voinnet *et al*. 1999), βC1 component associated with *Tomato yellow leaf curl China virus* [TYLCCNV: (Tb:Y10)] and *Tomato leaf curl Java virus* (ToLCJV) has been shown to suppress post transcriptional gene silencing and suppressor activity (Cui *et al*. 2005; Kon *et al*. 2007) as well as symptom induction requires βC1 nuclear localization (Cui *et al*. 2005). βC1 was also shown to bind to both ssDNA and dsDNA with size or sequence-specific manner. However, database searches reavealed no known proteins with discernible sequence relatedness to the βC1 protien. Zinc finger or Cys-His-rich regions (Krithi and Savithri, 2003) that might be involved in DNA binding were also absent, suggesting that the βC1 protein may contain a novel type of DNA-binding motif. Although how this relates to its function is not clear. As for many geminiviruses gene products, it is likely that βC1 will prove to be a multifunctional protein. The βC1 component causes cellular differentiation followed by vein swelling and greening and production of leaf like structure in cotton infected plants (Briddon and Markham 2000). It also can suppress gene silencing when transiently expressed in *Nicotiana benthamiana* (Mansoor *et al*. 2003). Further studies on the interactions between viral and host proteins could provide important insights into the movement mechanism of CLCuD. Preliminary evidence based on sequence analysis suggests that CLCuV-V1 protein has an arginine-rich region towards its N-terminal [1-29 amino acids] (Sharma *et al*. 2005b). Such localization signals has also been found in case of the CP of monopartite begomoviruses, like *Tomato yellow leaf curl virus*-Israel (Kunik *et al*. 1999), *Bhendi yellow vein mosaic virus* (Kumar *et al*. 2006).This region possibly may be responsible for the nuclear localization of CLCuV and an anti-NLS peptide-mediated resistance to leaf curl viruses could be exploited which is a new approach for integrated disease management. Knowledge of these mechanisms is important to understand pathogenesis and may help to develop control strategies to prevent CLCuD infection of plants.

CLCuV AND *B. tabaci* RELATIONSHIPS

The first report of tobacco whitefly, *B*. *tabaci* (Gennadius) transmission of this disease was reported by Golding (1930) and Kirkpatrick (1931). This disease is graft transmissible but not by seed and mechanical inoculation (Tarr 1951; Nour and Nour 1964; Cauquil and Follin 1983; Sharma 2002). There are very few reports on the virus-vector relationship (Nateshan *et al*. 1996; Sharma and Rishi 2003). Based on field data, it was noticed that the disease appeared at a late stage when the plants could tolerate the attack and gave good yield even in the presence of leaf curl disease. However, when crop was attacked at a young stage it suffered severely resulting in an almost complete loss of yield. The sowing dates are another important factor which play a decisive role in disease development and its spread. The Indian leaf curl causal agent is transmissible naturally only by its whitefly vector *B*. *tabaci* and recently it has been identified as B biotype of *B*. *tabaci* (Banks *et al*. 2001). Single *B*. *tabaci* is able to transmit the leaf curl agent (Sharma and Rishi 2003), but greater transmission efficiency is observed when a higher number *B*. *tabaci* (more than 10 whiteflies per plant) are present (Cauquil and Follin 1983). In experimental studies, 3.5 h acquisition access feeding (AAF) and 30 min inoculation access feeding (IAF) were required for transmission and the transmission threshold was 6.5 h (Kirkpatrick 1930, 1931). Males are significantly less efficient vectors than females and nymphs are as efficient as adults in acquiring the virus (Caciagli *et al*. 1995; Sharma and Rishi 2003). Viruliferous whiteflies can remain so for their entire life following a successful AAF; therefore a persistent, circulative relationship is postulated. Symptoms develop on inoculated plants within 15-30 days (Sharma 2002).

EPIDEMIOLOGY OF CLCuD

Practically no information is available on the development of CLCuD epidemics. Similarly scanty information is available on natural sources of primary and secondary infection

and further dissemination in field. Under northern Indian conditions, early sown cotton (1st week of April) had more disease incidence as well as insect population than late sown (3rd week of May) cotton. Therefore, practice of cotton sowing is discouraged in April. The progress of disease in general was maximum during the month of August as compared to July and September/October. Whiteflies are usually a problem in the mid to late season (August to October) and a significant positive correlation between the per cent disease incidence and whitefly count has been established. The data indicated that CLCuD is not seed-borne, and both the disease and insect vector must survive on reservoir hosts for further spread. Climate conditions (rainfall, wind, temperature) affect the epidemiology of CLCuD (Sharma and Rishi, 2004a). During 2000-2002 crop seasons CLCuD were observed in June and become severe in the month of July and August. The progress of disease slowed down later. The temperature varied between 43.2 ± 24.7°C, 43.4 ± 26.5°C, and 45.2 ± 23.6°C, respectively, and relative humidity (%) between 88.0-43.0, 83.0-35.0, and 85.0-333.0 that favored the disease incidence. Periods of rainfall prior to seed setting result in the development of a high population of the whitefly vector due to the abundance of food sources (Sharma 2002). Because cotton is grown only as a plant crop in a year (and without ratooning), alternate weed and cultivated hosts probably serve as virus reservoirs. Viruliferous whitefly populations typically infect cotton fields and primary sites of infection are established. Secondary spread to other cotton plants in the field probably occurs from those sites, and from additional vectors which enter the fields throughout the growing season (Giha and Nour 1969). The highest population levels of *B. tabaci*, and thus the disease incidence occur in October-November in northern India (Sharma and Rishi 2004a). In Pakistan also the highest vector population and disease incidence is seen in these months following August-September planting dates when cotton is most susceptible to damage (Giha and Nour 1969; Idris 1990).

MANAGEMENT STRATEGIES

Extensive efforts have been made to develop resistance to CLCuD in elite cotton varieties by both traditional breeding/selection and utilizing pathogen-derived strategies. However, as with any resistance strategy, a highly diverse pathogen is a negative indicator for the durability of resistance, suggesting that conventional resistance against CLCuD may not be long-lasting. Continued use of CLCuV-susceptible varieties without any program of their replacement constitutes a major risk for cotton production. So a premier focus should be given to eliminate the CLCuV disease and a well-planned program of evolving and introducing CLCuV-resistant (RS-875, LRA-5166 and LHH-144) and -tolerant (Om Shankar) varieties. Popular intra-hirsutum hybrids and AAH-1 and LDH-11 are high yielding. Intra-arboreum hybrids, of desired characteristics must be in place to gradually replace the existing CLCuV susceptible varieties (RST-9, HS-6, F-846, HS-777 etc). This is only the sole and the most promising and least expensive method of disease suppression.

Genetic variability in the begomoviruses inducing CLCuD has been demonstrated in Pakistan (Zhou *et al*. 1998) and India (Kirthi *et al*. 2004). In a strategy three popular varieties that became susceptible to CLCuD (HS-6, F-846, HS-777) were genetically modified using the *AV 2* gene of the most widely prevalent (>80%) *Cotton leaf curl Kokhran virus*–Dabawali (CLCuKV-Dab) in northern India; through *Agrobacterium*-mediated transformation (Sanjaya *et al*. 2005). Till now it has shown complete resistance up to T_3 seeds when challenge inoculated using whitefly under controlled condition (Dhawan *et al*. 2006).

Studies on integrated approaches using field sanitation, tolerant varieties, cultural practices (Dhawan *et al*. 2002), induced resistance (Verma *et al*. 1995) and field application of natural enemies of whitefly (Kirk *et al*. 2000) could be successfully exploited in bringing down the CLCuD incidence below economic thresh hold.

CONCLUSION AND FUTURE PROSPECTS

It has been confirmed that CLCuD is caused by two essential components, the begomovirus and a DNA-β satellite, in addition to a nanovirus-like DNA-1 component. The available evidence suggests that begomovirus complexes are expanding both in terms of their host range and geographical distribution. In the Indian subcontinent CLCuD continues to spread eastwards, threatening all the cotton growing regions of India and possibly even those of south eastern Asia. There is an urgent need of thorough disease mapping to know the distribution and diversity of these disease complexes and to assess their economic impact on agriculture. A major challenge is to elucidate how begomovirus and DNA-β gene products interact with each other and with plant signaling pathways to alter cell and tissue development. Geminiviruses and nanoviruses are currently being used to dissect DNA replication, cell-cycle control, tissue development and defense and counter-defense responses to biotic stress. It is anticipated that the unique attributes of DNA-β, particularly relating to the distinctive morphological changes induced during infection, will provide an additional means to investigate these processes in plants. Economic losses induced by geminiviruses continue to pose a significant threat to worldwide agricultural production, particularly to developing countries. Effective strategies of resistance will be predicted upon the development of a more complete understating of the components involved in host-virus-vector interactions. Biotechnological approaches can be used to evaluate the potential of these targets for engineering geminivirus resistant crops. Some major questions remain to be answered concerning begomovirus complexes. The mechanism of *trans*-replication of DNA. in absence of recognizable Rep binding sites and the apparently promiscuous nature of this satellite require investigation. With only a single type of DNA-β implicated in CLCuD in the Indian subcontinent, it is possible that a pathogen-derived resistance strategy aimed at the satellite would provide a more effective and durable control of the disease. A better understanding of the function and interactions of the host and viral components of the complex are essential to solve this problem.

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