Chapter 8

GEMINIVIRAL DISEASES OF COTTON

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INTRODUCTION

Plant viruses infect cotton in most parts of the world and can lead to decreased yield, or loss of the entire crop. While over 20 virus diseases of cotton have been described in the American Phytopathological Society "Cotton Disease Compendium" (Kirkpatrick and Rothrock, 2001), only a few have actually been shown to be of virus etiology. The main viruses, for which a causative relationship has been proven, include several geminiviruses of the genus *Begomovirus* (Briddon and Markham, 2001; Idris and Brown, 2004) and a luteovirus belonging to the genus *Polerovirus* (Corrêa *et al*., 2005; Distéfano *et al*., 2010; Silva *et al*., 2008). Among these the geminiviruses are the most destructive and a potential threat to cotton cultivation all over the world. The ubiquitous presence of white fly (*Bemisia tabaci Gennadius*), the insect vector of begomoviruses in all major cotton production areas, and human activity that has disseminated these viruses into geographical locations where they were not found earlier has compounded the problem. Geminiviruses are single-stranded (ss)DNA viruses with small circular genomes encapsidated in characteristic twinned (geminate) particles that are transmitted by insect vectors. They infect either monocotyledonous or dicotyledonous plants and are taxonomically divided into four genera based on insect vectors, genome organization and host range (Stanley *et al*., 2005). Geminiviruses that are transmitted by *Bemisia tabaci*, are classified in the genus *Begomovirus*. These are the most numerous and the most important due to their emergence as a major limiting factor in the production of many dicotyledonous crops, including cotton, in the warmer parts of the World (Seal *et al*., 2006). Begomoviruses may be further divided into two distinct groups, those originating from the Old World (OW) and those prevalent in the New World (NW). Begomoviruses from the NW have genomes consisting of two genomic components, known as DNA A and DNA B (each 2600 – 2800 nucleotides [nt]), and both are required to systemically infect plants. In the OW, although a few bipartite begomoviruses have been identified, the majority of begomoviruses are monopartite, with a genome consisting of a single circular ssDNA component homologous to the DNA a component of the bipartite begomoviruses (Fig.1). Furthermore, the majority of monopartite begomoviruses are associated with additional small (approx. 1350 nt) circular ssDNA molecules. The first is a satellite, known collectively as betasatellites, which is often required by the helper begomovirus to successfully infect host plants and induce disease symptoms. The second is satellite-like molecule, collectively named alphasatellites, which is not essential for the virus to infect plants. The two viral diseases of cotton of confirmed begomovirus etiology are cotton leaf curl disease (CLCuD)

(Fig. 2) and cotton leaf crumple disease (CLCrD). Cotton leaf curl disease has been reported from the Indian subcontinent and Africa affecting tetraploid cotton (*Gossypium hirsutum* and *G. barbadense*) introduced from the Old World while diploid cottons, that have their origins in the OW, are completely immune to the disease. Cotton leaf crumple is found in the Americas and is, in most years, not a significant problem.

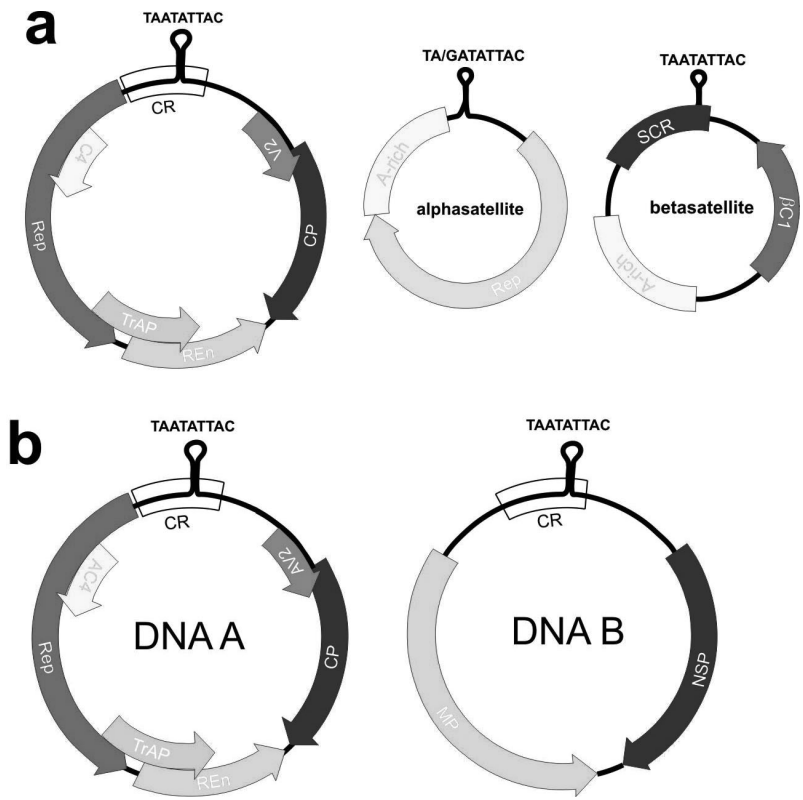

Figure 1. Arrangements of the genomes of bipartite (a) and monopartite begomoviruses with their associated betasatellites and alphasatellites (b).The positions and orientations of genes are shown by arrows. The genes encode the coat protein (CP), the replication-associated protein (Rep), the transcriptional activator protein (TrAP), the replication enhancer protein (REn), the movement protein (MP) and the nuclear shuttle protein (NSP). The products encoded by open readings frames (A)V2 and (A)C4 have yet to be named. Characteristically, begomoviruses native to the New World lack the AV2 gene. The alphasatellites encode a Rep whereas the single gene encoded by the betasatellites, which is encoded in the complementary-sense, is known as βC1. Bipartite begomoviruses contain a sequence of ~200 nt which is conserved between the DNA A and DNA B components and is known as the common region (CR) The hairpin structure is shown as position zero for each component. This contains the nonanucleotide sequence, which is highly conserved.

COTTON LEAF CURL DISEASE

A phylogenetic tree based on complete nucleotide sequences of all cotton begomoviruses is shown in Figure 3. Cotton leaf curl is a destructive disease of cotton and several other malvaceous plant species that is transmitted by *B. tabaci*. Presently the disease is prevalent throughout Pakistan and northwestern India. Infected cotton plants display a range of symptoms such as leaf curling, stunting and a poor yield of cotton fiber. Besides, affected plants may develop enations on the veins on the undersides of leaves which may develop into cup-shaped, leaf-like structures (Fig. 2). Symptoms in cotton usually appear within 2–3 weeks of inoculation by *B. tabaci* (Singh *et al*., 1997) and are primarily characterized by a deep downward cupping of the youngest leaves. This is followed by either upward or downward curling of the leaf margins, swelling and darkening of the veins as well as the formation of enations on the veins, which frequently (dependant on variety) develop into cup-shaped, leaf-like structures.

Figure 2. Symptoms induced by cotton leaf curl disease in cotton. Note the vein swelling, vein darkening (often CLCuD affected plants appear darker green than non-affected plants) and enations on the veins. Frequently these enations develop into cup-shaped leaf-like structures. In this case the leaves show upward leaf curling. However, downward curling may also occur.

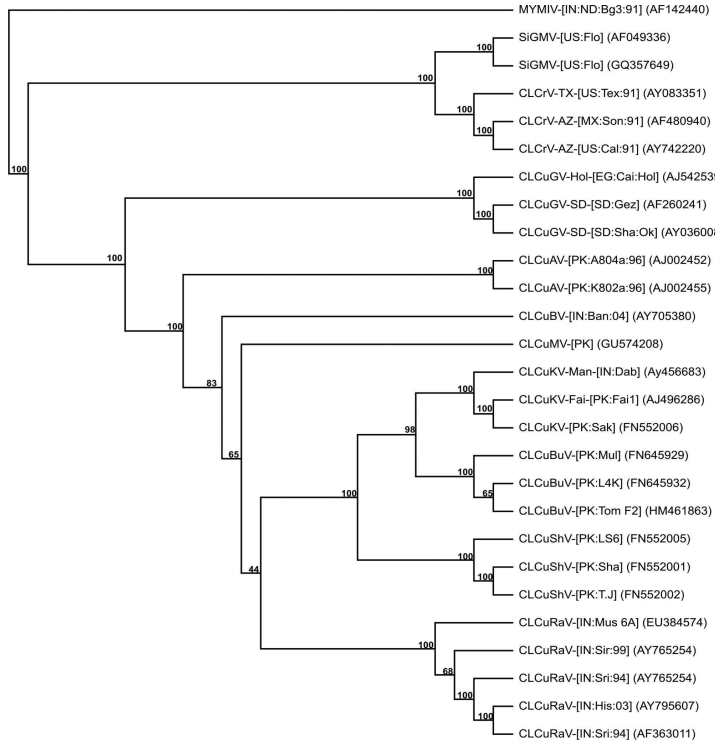

Figure 3. Phylogenetic dendrogram, based upon an alignment of the full length genome (or DNA A genomic component) sequences of selected begomoviruses. The figures at nodes indicate percentage bootstrap confidence values (1000 replicates). The viruses shown are Cotton leaf crumple virus (CLCrV), Cotton leaf curl Alabad virus (CLCuAV), Cotton leaf curl Burewala virus (CLCuBuV), Cotton leaf curl Gezira virus (CLCuGV), Cotton leaf curl Kokhran virus (CLCuKoV), Cotton leaf curl Multan virus (CLCuMuV), Cotton leaf curl Rajasthan virus (CLCuRaV), Cotton leaf curl Shadadpur virus (CLCuShV) and Sida golden mosaic virus (SiGMV). The tree was rooted on an outgroup, the DNA A component of Mungbean yellow mosaic virus (MYMV), a bipartite begomovirus that occurs in southern Asia and is only distantly related to the remaining monopartite viruses. Of the species shown, only MYMV and SiGMV do not cause disease in cotton. The geographical origins of the viruses are indicated. In each case the database accession number of the sequence used is given. (Isolate descriptors are as given in Fauquet *et al*., 2008).

CLCuD has been recorded from several countries in Africa. In fact it was first named as leaf curl by Kirkpatrick (Kirkpatrick, 1931) who also described the symptoms of the disease as definite curling of the leaf margins, either upward or downward and a peculiar crinkled appearance (enations) may be produced by the veins. Veins of the leaves become thickened which are more pronounced on the underside. Two types of vein thickening are commonly seen, small vein thick-

ening (SVT) and main vein thickening (MVT). Small vein thickening is more common in field conditions and is characterized by small green bead-like thickening on the young leaves. These irregular thickenings gradually extend and coalesce to form a continuous reticulation of the small veins. Main vein thickening is characterized by the green thickening of the distal ends of the larger veins of young leaves. The thickening first appears near the leaf margin and then extends inward to form a network of dark green thickened main vein (Watkins, 1981). In extreme but not infrequent cases, formation of the cup- shaped, leaf-like outgrowths appear on the underside of the leaves. Enations has however been observed even on leaves of plants with only mild bead-like vein thickening (Mahmood, 1999). According to Tarr (1951), severely infected plants may show spirally twisted petioles, fruiting branches and, to a lesser extent, the main stem, which tends to grow tall with elongated internodes in *Gossypium barbadense*. All the varieties show a dwarfing effect, more so in the dwarf varieties, internodal distance is reduced and the affected plants become stunted in early infection with adverse effect on fruiting. There is reduction in boll number and boll weight resulting in loss of yield. In extreme cases, the plants succumb to its attack and some growers had to plough-up their crop during 1991-1992 in Pakistan.

History of Cotton Leaf Curl Disease

In Africa, CLCuD was first reported 1912 from Nigeria affecting *G. barbadense*. In 1924, it was recorded in Sudan and subsequently from Tanzania 1926 (Kirkpatrick, 1931). It was one of the most important diseases of cotton in these countries and had potential to cause significant losses. On the Indian subcontinent CLCuD was first observed near Multan in 1967 (Hussain and Ali, 1975) on a few individual plants and has been noted consistently since then. In the beginning, the disease did not attract serious attention because it was sporadic and of minor economic importance. The disease, however, become prominent in 1973 when it was observed on several varieties, including 149-F and B-557, with incidences of 5% of the field. The disease occurred only late in the season on the upper portion of the plant. Hussain and Mahmood (1988) reported that in 1987, the incidence was up to 80% in certain fields. In 1988, the disease damaged the cotton crop on 60 hectares in the Multan district. In the following years the affected area increased. It affected 200 hectares in 1989 and 800 hectares in 1990. The incidence increased substantially and caused losses from 22.3% to 68.5% in the affected fields in some areas of Punjab depending upon the variety, time of infection and environmental conditions. However, in 1991, the disease reached epidemic proportion, affecting an area of 14,000 hectares in Multan, Khanewal and Vehari Districts. In 1992, the disease spread to more than 48,500 hectares causing a decrease in production and significant monetary loss to the country. In 1993, the disease spread to the entire cotton belt of the Punjab with varying intensity causing losses across 889,000 hectares. The disease was also reported from Dera Ghazi Khan district of Punjab and Sindh province, during 1996-97. The loss in yield varied with the intensity of the disease and with the crop stage at which it occurred. In the severest cases, farmers were forced to plough-up their fields. Cotton production in Pakistan decreased from 1.938 million metric tons in 1991 to 1.445 million metric tons in 1992 and fell further to 1.105 million metric tons in 1993. CLCuD was the main force behind yield decline in these years. The first 3 years of the disease epidemic (1992-1994) in Pakistani Punjab were the most severe in terms of disease intensity. The epidemic of CLCuD in Pakistan is one of the best examples of the dramatic shift in importance of a previously insignificant endemic disease.

The introduction of resistant cotton varieties in the late 1990s (as described later) restored cotton production in Pakistan to above the levels seen before the epidemic of CLCuD. However, during 2001 typical disease symptoms were seen in resistant cotton varieties, suggesting the appearance of a resistance breaking strain of CLCuD (Mansoor *et al*., 2003a) which has now spread into northwestern India.

Spread of the Disease by the Insect Vector

CLCuD is transmitted by feeding of the whitefly (*B. tabaci*) which can complete the entire cycle, from the acquisition of the virus to infection of a new host plant, within 6.5 hours. The disease is not mechanically transmissible and is not carried in soil or seed. *B. tabaci* is the only known vector of begomoviruses (Brown, 1997). *B. tabaci* is capable of establishing high population levels, particularly in crops grown under irrigated, arid conditions in both field and greenhouse systems. In addition, this whitefly has the potential to colonize a wide range of dicotyledonous species, among which are primarily vegetables and fiber species of great importance to worldwide agricultural production. Recent studies indicate that there are numerous populations of *B. tabaci* that vary somewhat in their capacity to develop high population densities and cause feeding damage within their host ranges and the efficacy with which they can transmit geminiviruses (Bedford *et al*., 1994; Brown and Bird, 1992; Brown *et al*., 1995; Maruthi *et al*., 2002).

Aetiology of CLCuD

The aetiology of CLCuD from Sudan and the Indian subcontinent has been determined. In both regions the disease is caused by begomovirus complexes consisting of monopartite begomoviruses, a disease-specific, symptom determining satellite and frequently also involves an additional satellite-like molecule.

Diversity of Monpartite Begomoviruses Associated with CLCuD

Cotton leaf curl disease (CLCuD) occurring on the Indian subcontinent has been shown to be associated with several begomoviruses (Harrison *et al*., 1997; Kirthi *et al*., 2004; Mansoor *et al*., 1993; Mansoor *et al*., 2003b; Nadeem *et al*., 1997; Zhou *et al*., 1998). Initial studies of the disease in Pakistan in the early 1990s determined that the disease was associated with begomoviruses (Mansoor *et al*., 1993a). At that time only bipartite and monopartite begomoviruses were known, the satellites associated with some monopartite begomoviruses were not identified until 1999-2000 (Briddon *et al*., 2001; Mansoor *et al*., 1999). No evidence for the presence of a DNA B component was found, leading to the conclusion that the disease was caused by a monopartite begomovirus, yet the monopartite begomovirus identified (now known as Cotton leaf curl Multan virus [CLCuMV]) was experimentally only poorly infectious to cotton and did not induced the symptoms typical of CLCuD (Briddon *et al*., 2000). This indicated that some component or factor, essential for induction of the disease symptoms, remained to be identified. The fact that the disease was shown experimentally only to be transmissible by *B. tabaci*, the vector of begomoviruses, strongly suggested that the additional component must consist of ssDNA.

An early study into the diversity of begomoviruses associated with CLCuD concluded that there were essentially four begomovirus variants infecting cotton in Pakistan (Zhou *et al*., 1998). Three of the viruses identified are now classified as species; CLCuMV, Cotton leaf curl Alalabad virus (CLCuAV) and Cotton leaf curl Khokhran virus (CLCuKV). Further investigation identified additional species – Papaya leaf curl virus (Mansoor *et al*., 2003b) and Tomato leaf curl Bangalore virus (Kirthi *et al*., 2004). In northwestern India many of the viruses identified in Pakistan were subsequently found to be present and an additional distinct species, Cotton leaf curl Rajasthan virus (CLCuRaV) was also identified (Kirthi *et al*., 2004). This species was later also identified in Pakistan infecting both cotton and tomato (Nawaz-ul-Rehman *et al*., 2010; Shahid et. al., 2007).

More recently the genetic make-up of begomoviruses in Pakistan has changed dramatically. The virus associated with resistance breaking in cotton across Pakistan has been shown to be a distinct recombinant begomovirus, Cotton leaf curl Burewala virus (CLCuBuV) (Amrao *et al*., 2010b). This virus consists of sequences derived from two of the begomovirus species associated with the CLCuD epidemic during the 1990s, CLCuMuV and CLCuKoV. Surprisingly this virus lacks one of the usual complement of genes encoded by begomoviruses, as will be discussed later. As was the case with the epidemic in the 1990s, the virus associated with resistance breaking in cotton (CLCuBuV) has spread into India (Kumar *et al*., 2010) and there are now problems with CLCuD in previously resistant varieties with particularly severe losses to the crop during 2009-2010.

Throughout the epidemic of CLCuD in most of Pakistan during the 1990s, the cotton growing region of southern Sindh province remained largely unaffected by the disease. For this reason, the farmers there were not growing resistant cotton varieties. However, during 2003-2004 the disease appeared in central and lower Sindh, causing substantial yield losses. This coincided with introduction of cotton varieties not approved by the Government authorities for cultivation, since they are highly susceptible to CLCuD. Analysis of the begomoviruses associated with the outbreak has shown the presence in Sindh of CLCuKoV and a newly identified recombinant species for which the name Cotton leaf curl Shadadpur has been proposed (Amrao *et al*., 2010a). The reason for the differences between Sindh and the rest of Pakistan, with respect to the incidence of CLCuD and the diversity of associated begomoviruses, remains unclear. However, the presence in Sindh of a distinct biotype of *B. tabaci*, with possible distinct host ranges and virus vectoring specificities, has been suggested.

In Sudan, where cotton production was severely affected by CLCuD during the early parts of the 20th century, a single begomovirus species (Cotton leaf curl Gezira virus) has been shown associated with CLCuD in Africa (Idris and Brown, 2002). The virus has been characterized from several malvaceous hosts including cotton, okra and *Sida alba*. Recent investigation into diversity revealed that limited diversity exist in cotton begomoviruses in Africa. A related but distinct species has been reported from hollyhock named as Hollyhock leaf crumple virus. These viruses are only distantly related to CLCuD begomoviruses found in the Indian subcontinent, being instead more closely related to other begomoviruses originating from Africa and the Mediterranean region. These results suggest that distinct begomovirus complexes were mobilized from indigenous hosts to susceptible cotton upon their cultivation

in Africa and Asia. Nevertheless, the begomovirus components from Asia and Africa complement each other under experimental conditions (R.W. Briddon, unpublished data). Thus, human activity may disseminate these begomoviruses within the Old World where begomovirus components have been reported.

DNA Satellites Associated with CLCuD

Satellites are defined as viruses or nucleic acids (DNA or RNA) that depend on a helper virus for their replication but lack extensive nucleotide sequence identity to their helper virus and are dispensable for its proliferation (Murant and Mayo, 1982). The majority of satellites consist of RNA and are associated with viruses with RNA genomes and have no discernable effects on the symptoms caused by their helper viruses in plants (Hu *et al*., 2009). However, some satellite module symptoms, either ameliorating or exacerbating the symptoms induced by the helper virus.

The first ssDNA satellite was identified in association with Tomato leaf curl virus (ToLCV) in tomatoes from Australia (Dry *et al*., 1997). The satellite, known as the ToLCV-sat, is a circular molecule of approx. 700 nucleotides that has little similarity to its helper virus other than a predicted hairpin structure containing within the loop the sequence TAATATTAC (the so called nonanucleotide motif) that (for geminiviruses) forms part of the virion-strand origin of replication). This molecule has no discernable effect on ToLCV infections and encodes no proteins. At the time this was a novel oddity. Its significance, being related to a much larger group of begomovirus-associated satellites, was not realized until later.

Alphasatellites Associated with Cotton Leaf Curl Disease

The search for additional components associated with begomoviruses that cause CLCuD first identified a class of molecules that were named DNA 1 and that we now refer to as alphasatellites (Mansoor *et al*., 1999). The alphasatellites comprise a group of closely related ssDNA molecules that encode a single protein, a rolling-circle replication initiator protein (the replication-associated protein [Rep]). As a consequence, alphasatellites are capable of autonomous replication in cells of host plants. Since, by definition, satellites depend on a helper virus for their replication, alphasatellites are best described as satellite-like. For all other functions alphasatellites depend on their helper begomoviruses, including movement within plants and insect transmission between plants (Mansoor *et al*., 1999; Saunders *et al*., 2002; Saunders and Stanley, 1999). This likely requires the alphasatellite ssDNA to be encapsidated within the coat protein of the helper virus.

Alphasatellites share no significant levels of sequence identity to geminiviruses but contain a predicted hairpin structure with, in the loop, the nonanucleotide sequence. Surprisingly, the alphasatellite Rep exhibits high levels of sequence identity to the Reps encoded by components of nanoviruses (Saunders *et al*., 2000; Saunders and Stanley, 1999). The family Nanoviridae is a second family of DNA viruses with circular single-stranded genomes that replicate by a rolling-circle mechanism (Gronenborn, 2004). Their genomes are multipartite,

consisting of 6 to 8 components that are encapsidated is small icosahedral particles and are transmitted plant-to-plant by aphids. It has been suggested that begomoviruses may have captured a nanovirus Rep encoding component during a co-infections of a nanovirus and a begomovirus (Mansoor *et al*., 1999; Saunders *et al*., 2002; Saunders and Stanley, 1999). The benefit, to the helper begomovirus, of the presence of an alphasatellite remains unclear. Initially it was suggested that the alphasatellite may act as a "dampener", mopping up cellular resources and ameliorating the symptoms induced, thus extending the life of the plant and thereby benefitting the virus by extending the period during which it may be transmitted by the insect vector to new hosts. Although some evidence in support of this has been forthcoming (Wu and Zhou, 2005), evidence recently obtained suggests that the Rep encoded by alphasatellites may be a suppressor of host silencing (post-transcriptional gene silencing [PTGS], also known as RNA interference [RNAi]) and thus involved in overcoming host defences (Nawaz-ul-Rehman *et al*., 2010). RNAi is, amongst its many functions, an RNA induced defence mechanism that is involved in the destruction of foreign and aberrant RNA (Voinnet, 2001; Voinnet *et al*., 1999).

Interestingly, some nanovirus infections are associated with multiple Rep encoding components. In addition to the *bona fide* virus Rep encoding component, known as the master Rep component (Timchenko *et al*., 1999; Timchenko *et al*., 2000), there are additional components that are not related to the components of the virus. These molecules are satellite-like and may be the form of the progenitor of the begomovirus-associated alphasatellites that was originally captured during a co-infection. A proposal is being prepared to classify these molecules with the begomovirus satellite-like components as alphasatellites (R.W. Briddon, manuscript in preparation). The begomovirus-associated satellite-like molecules differ little from those associated with nanoviruses other than being larger in size (by 300-400 nt). Most of this size increase is due to the presence, in the begomovirus-associated satellite-like molecules of a sequence rich in adenine (the A-rich region: Fig. 1). This is believed to be required for effective encapsidation of the molecule in the begomovirus coat protein. Begomoviruses have a strict size selection for movement in plants and encapsidation; selecting for unit length (~2800 nt; begomovirus genomes and genomic components), half unit length (~1400 nt; alphasatellites, betasatellites defective molecules derived from the virus genome) and one quarter unit length (~600-700nt; the ToLCV-sat and defective molecules derived from the virus genome) (Frischmuth *et al*., 1997; Frischmuth *et al*., 2001; Frischmuth and Stanley, 1991; Rojas *et al*., 1998).

Betasatellites Associated with CLCuD

The identification of an alphasatellite associated with CLCuD affected cotton spurred the search for further half-unit length ssDNA molecules. This led to the identification of a diverse set of molecules which we now call betasatellites (Briddon *et al*., 2008). Betasatellites are approximately half the size of their helper virus genomes (~1350 nt) and have a highly conserved structure, despite the fact that their sequences may show as little as 45% nucleotide sequence identity (Briddon *et al*., 2003; Bull *et al*., 2004) They encode a single gene (known

as βC1) in the complimentary sense, have a sequence rich in adenine and sequence of 80-100 nt that is highly conserved between all betasatellites so far identified (Briddon *et al*., 2003; Briddon *et al*., 2001)(Fig. 1).

The Rep encoded by geminiviruses is a sequence specific DNA binding protein. The protein binds to specific sequence motifs, known as iterons, adjacent to the nonanucleotide-containing stem loop structure, to initiate virion-strand viral DNA replication. The iterons of geminivirus species differ, meaning that the Rep of one species will not recognise the origin of replication (iterons) of another species. The DNA A and DNA B components of bipartite begomoviruses share a sequence of high sequence identity, known as the common region, that encompasses the origin of replication (iterons and stem-loop structure). This serves to maintain the integrity of the split genome, allowing the DNA A-encoded Rep to initiate rolling circle replication of both components. Although transreplicated by the Rep encoded by their helper begomoviruses, betasatellites do not contain the iterons sequences of their helper begomoviruses, rasing the question of how the interaction functions. A single virus may transreplicate numerous betasatellites (for example the begomovirus Ageratum yellow vein virus can transreplicate the majority of betasatellites tested; Briddon *et al*., 2003) and a single betasatellite may be transreplicated by numerous distinct begomovirus species (as is the case for Cotton leaf curl Multan betasatellite [CLCuMB] that is associated with CLCuD across the Indian sub-continent; Mansoor *et al*., 2003b). This indicates that betasatellites have a much looser relationship with their helper viruses than the DNA B components of bipartite begomoviruses have with their cognate DNA A components. Although far from resolved, recent evidence suggests that betasatellites contain a hyper-variable region of sequence lying between the SCR and the A-rich region. This vairable sequence contains (in most cases) numerous sequences that are similar to (differing by only a few nucleotides) the iterons of begomoviruses. It is possible that the sepseud-iterons allow betasatellites to interact with the Rep proteins of multiple begomoviruses for their replication. In the field this relaxed relationship leads to frequent exchanges of betasatellites between distinct begomoviruses (as has been proposed for CLCuD where a single betasatellite [CLCuMB] is capable of interacting with numerous begomoviruses to induce the disease; Mansoor *et al*., 2003b) and the presence in some plants of multiple betasatellites apparently maintained by single begomoviruses (Mubin *et al*., 2010). Overall this means that betasatellite-associated begomoviruses may rapidly adapt to changing conditions by interacting with different betasallites.

In contrast to the alphasatellites, for which there is some indication of their possible evolutionaty origins, the orgins of betasatellites remain unclear. There are no sequences with significant sequence similarities in the databases. However, the presence in betasatellites of an A-rich sequence suggests that, like alphasatellites, they may have originated with another group of, as yet unidentified, single stranded circular DNA replicons.

Since they were first identified, research on betasatellites, to identify possible functions encoded by this satellite, has moved at a rapid pace. As well as being required (by some begomoviruses, including those associated with CLCuD) to infect the host plants from which they were isolated and induce typical disease symptoms, they were shown in some cases to elevate virus DNA levels (Briddon *et al*., 2001; Saunders *et al*., 2000). This suggested either that the

betasatellite enhanced virus replication (more viral DNA per infected cell) or that the betasatellite enhanced virus movement in plants (thus more cells infected). So far all functions of betasatellites have been attributed to the product of the single gene they encode, known as βC1 (Fig. 1). βC1 is a pathogenicity (symptom) determinant (Saeed *et al*., 2005; Saunders *et al*., 2004), a suppressor of PTGS, may facilitate virus movement (Saeed *et al*., 2007), binds DNA (Cui *et al*., 2005), and interacts with a variety of host and virus encoded factors including a host ubiquitin-conjugating enzyme (part of the host ubiquitin proteasome pathway that is involved in protein turnover)(Eini *et al*., 2009), ASYMMETRIC LEAVES 1 (a host factor involved in controlling leaf development) (Yang *et al*., 2008), attenuates the expression of jasmonic acid responsive genes implicated in plant defence against insects (suggesting that βC1 may enhance virus transmission by making the plant more "palatable" for the vector; Yang *et al*., 2008) and the helper virus coat protein (Kumar *et al*., 2006). Recently studies of a βC1 protein have shown that it has the capacity to self-interact and form higher order multimers *in vitro* and *in vivo* (Cheng *et al*., 2011). Mutant βC1 proteins that lack the capacity to self-interact, and that do not form multimers, were also unable to induce typical symptoms in plant, suggesting that βC1 acts, *in planta*, as a multimer. However, the precise significance of this finding remains unclear.

In addition to being shown to be the dominant pathogenicity determinant in begomovirus-betasatellite infections, expression of the βC1 of Cotton leaf curl betasatellite from a Potato virus X (PVX) vector, has shown that this is able to induced all the symptoms typical of CLCuD in tobacco in the absence of all helper virus encoded factors (Qazi *et al*., 2007). Constitutive expression of CLCuMB βC1 in transgenic plants under the control of the Cauliflower mosaic virus 35S promoter induces virus-like symptoms but these do not resemble typical CLCuD symptoms. Since PVX, in common with the begomoviruses that cause CLCuD, is phloem limited, this indicates that βC1 determines symptoms, but the virus contributes by ensuring the gene is expressed in the correct tissues.

CLCuD occurring in Pakistan during the 1990s, although associated with multiple distinct begomovirus species, involved only a single species of betasatellite (CLCuMB). However, following resistance breakdown in cotton during the early 2000s, a distinct variant of CLCuMB became prominent (referred to as the Burewala strain of CLCuMB [CLCuMB^{Bur}]), with the earlier variant (referred to as the Multan strain of CLCuMB [CLCuMB^{Mul}) no longer encountered (Amin *et al*., 2006). CLCuMB^{Bur} differs from CLCuMB^{Mul} in containing some sequence (~80 nt) in the SCR derived from a tomato betasatellite. The significance of this recombinant sequence remains unclear but is characteristic of the resistance breaking strain of CLCuD. The recombinant betasatellite CLCuMB^{Bur} was earlier detected in tomato from India and indicates a close relationship between the begomovirus diseases of cotton and tomato.

CLCuD in Sudan is similarly associated with a betasatellite (Idris *et al*., 2005). This betasatellite, Cotton leaf curl Gezira betasatellite (CLCuGB), is distinct from that occurring on the Indian subcontinent (Figure 4). CLCuGB may be transreplicated and maintained by CLCuMV to induce typical disease symptoms. Interestingly, CLCuGB is widespread across Africa and, together with distinct begomoviruses, causes disease in other species, including okra (Kon *et al*., 2009) and the non-malvaceous crop tomato (Chen *et al*., 2009). This contrasts with the situ-

ation on the Indian sub-continent. Although CLCuMB is occasionally identified in other plant species, it is only consistently found in ornamental *Hibiscus* and the fiber crops *Hibiscus cannabinus* and *Hibiscus sabdariffa* (Das *et al*., 2008; Paul *et al*., 2008; Roy *et al*., 2009). Disease in, for example, okra (Jose and Usha, 2003), chillies (Hussain *et al*., 2009) and tomato (Sivalingam *et al*., 2010) are associated with distinct betasatellites.

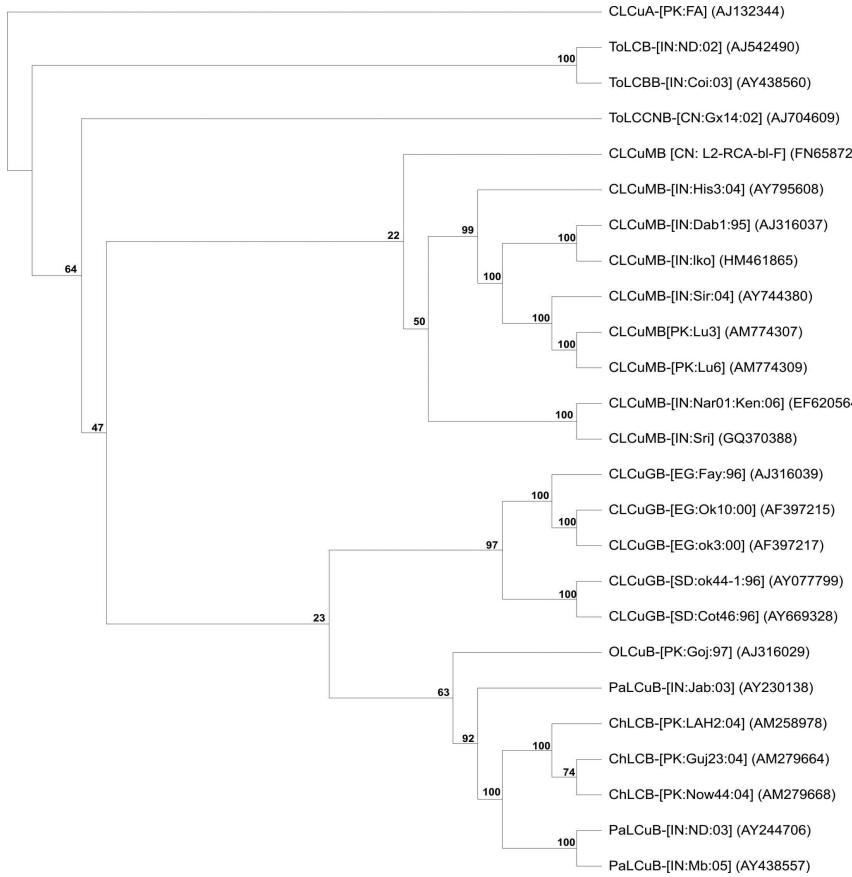

Figure 4. Phylogenetic dendrogram, based upon an alignment of the full length sequences of selected betasatellites. The figures at nodes indicate percentage bootstrap confidence values (1000 replicates). The betasatellites shown are Chilli leaf curl betasatellite (ChLCB), Cotton leaf curl Multan betasatellite (CLCuMB), Okra leaf curl betasatellite (OLCuB), Papaya leaf curl betasatellite (PaLCuB). The tree was rooted on an outgroup, the cotton leaf curl alphasatellite (CLCuA); an unrelated sequence of a similar size.. The geographical origins of the betasatellites involved in CLCuD (CLCuMB and CLCuGB) are indicated. In each case the database accession number of the sequence used is given. (Isolate descriptors are as given in Briddon *et al*., 2008).

COTTON LEAF CRUMPLE DISEASE

Cotton leaf crumple disease (CLCrD) is a disease of cotton that occurs in the New World. CLCrD was first reported from California (Dickson *et al*., 1954) and in Arizona a few years later (Allen *et al*., 1960). The symptoms of the disease are characteristically floral distortion, hypertrophy of interveinal tissue resulting in downward curling of leaves, and a foliar mosaic accompanied by vein clearing and frequent vein distortion (Brown and Nelson, 1987). Losses resulting from CLCrD infection range from 21 to 86%, depending on the age of plants at the time of infection (Allen *et al*., 1960; Brown *et al*., 1987; van Schaik *et al*., 1962). CLCrD can be transmitted experimentally by *B. tabaci* to numerous species within the families Malvaceae and *Fabaceae* families (Brown and Nelson, 1987). The disease mainly occurs in the Sonoran Desert of Arizona and Sonora, Mexico. The disease also occurs in southern California, the Rio Grande Valley of Texas, and Guatemala. The disease is among the earliest known where the causal agent was suspected to be a begomovirus.

Although geminate virus particles typical of geminiviruses were observed in CLCrD-affected cotton in the early 1980s (Brown and Nelson, 1984), the complete sequence of the virus concerned (Cotton leaf crumple virus [CLCrV]) was not determined until 2004 (Idris and Brown, 2004).

CLCrV is a typical bipartite begomovirus that characteristically (for New World begomoviruses) lacks the V2 gene (Fig. 1). It is closely related to other begomoviruses occurring in the New World. The DNA A component shares the highest levels of nucleotide sequence identity with Squash leaf curl virus, whereas the DNA B component has the highest levels of identity with Abutilon mosaic virus and Bean calico mosaic virus. CLCrV is only distantly related to the viruses causing CLCuD in the Old World.

MANAGEMENT OF BEGOMOVIRUS DISEASES OF COTTON

The diploid species of cotton (*G. arboreum* and *G. herbaceum*), that were grown across Asia and Africa prior to the introduction of tetraploid cottons (*G. hirsutum* and *G. barbadense*) are immune to CLCuD. A recent study on cotton species grown in a living herbarium being maintained at CCRI Multan has identified other sources of resistance in wild species of cotton (Azhar *et al*., 2010). The major obstacle however, is the ploidy barriers and therefore several steps are necessary to introduce characters from diploid to tetraploid cotton. The task has been complicated due to the lack of understanding of mechanism of resistance in diploid Asiatic species and the lack of DNA markers linked to disease resistance. Two strategies have been employed to incorporate useful characters from *G. arboreum*; one is the introduction of useful traits from *G. hirsutum* into *G. arboreum* (often termed as hirsutization of *G. arboreum*) and the other is to clone useful genes from *G. arboreum*. However, both strategies require long-term commitment.

During early 1990s, to counter the first epidemic of CLCuD, conventional selection and breeding was used to identify existing *G. hirsutum* cultivars in Pakistan with resistance to the disease and transfer this resistance to other, elite varieties. Varieties CP-15/2 and LRA-5166 were identified with stable resistance to the disease. Efforts concentrated on hybrids having

these two elite varieties as parents. The progenies 1098 and 1100, out of the cross 492/87 x CP-15/2, in the crop season 1992-93 emerged as the first instalment of lines resistant to CLCuD. Ali (1997) laid out a study to determine the mode of inheritance of host plant resistance mechanism against CLCuD. Crosses were made between the most susceptible cotton genotype, S-12 and the resistant variety, LRA-5166. Their F1s and backcross to LRA-5166 showed complete resistance against the disease. The F2 segregating population showed good fit to a ratio of 3:1 resistant/ susceptible. Thus, it was concluded that the disease is under the control of single dominant gene. It was reported that F1s of a cross between resistant parent (CIM-443) and susceptible parent (CIM-240) were often tolerant. He further observed that the cross between two tolerant parents produced a resistant F1 with one dominant gene coming from each parent. Thus it was indicated that two dominant genes governed resistance against the CLCuD. On the other hand, Rahman (Rahman *et al*., 2002) screened 22 genotypes of cotton for resistance against CLCuD. Out of these 22 genotypes only six, LRA-5166, Cedix, FVH-53, CIM-1100, CP-15/2 and CIM-443, were found to be extremely resistant. The resistance sources (LRA-5166 and CP-15/2) were employed for crosses with the most susceptible variety, S-12 (Rahman *et al*., 2005). The plants in the F_2 generation of crosses S-12 X LRA-5166, S-12 X CP15/2 and S-12 X CIM-443 and their reciprocals demonstrated a 13:3 (non-susceptible:susceptible) ratio. However, on the basis of F_3 progeny test, he suggested that two dominant genes at two loci acting epistatically might have conditioned the CLCuD resistance and a third gene known as suppressor gene is also involved which inhibits the expression of major genes. In spite of substantial efforts, advancement in breeding cotton for resistance to CLCuD has been slow. The main bottleneck is that the breeders have had to rely on field inoculation by whiteflies to screen for resistance.

Further efforts, involving crosses between local varieties and exotic virus resistant cultivars at the Central Cotton Research Institute, Multan (Pakistan) led to the development of several CLCuD resistant varieties. Subsequently further inbred lines, including Cedix, MS-40 and Reba, were found to be resistant to the disease. It has been shown that the resistance of Cedix, a cotton cultivar highly resistant to CLCrV from El Salvador, is controlled by two dominant and supplementary genes, which must occur together in order to confer full resistance (Wilson and Brown, 1991). Recently, more efforts were made to find resistance in *G. hirsutum* to CLCrV.

In the late 1990s, the widespread use of resistant varieties essentially removed CLCuD as a significant factor in cotton production in Pakistan. However, during the 2001 cropping season, symptoms of CLCuD appeared on all previously resistant cultivars at Burewala, district Vehari and by 2002 the disease reached epidemic proportions. This indicated the emergence of a resistance breaking strain of the virus (Mansoor *et al*., 2003a). Recently the begomovirus complex associated with resistance breakdown has been characterization. The so called "Burewala" resistance breaking strain of CLCuD is associated with a novel recombinant begomovirus, CLCuBuV, that lacks one of the usual complement of genes encoded by begomoviruses, C2 (Amrao *et al*., 2010b). The C2 protein has, amongst other functions, a suppressor of gene silencing activity. This may suggest that resistance breaking is due to the lack of C2, in turn suggesting that host resistance, in resistant cotton varieties, is due to recognition of the C2 protein (the so called avirulence determinant recognised by the host encoded resistance gene). However, this hypothesis has yet to be tested and it remains possible that resistance breaking is due to the

recombinant betasatellite (mentioned above) associated with the Burewala strain of CLCuD. At this time no reliable source of resistance to the resistance breaking strain of the disease has been identified and efforts are mainly concentrated on the transfer of resistance from diploid sources.

A number of reports on the resistance in *G. barbadense* to CLCuD have been published. Hutchinson and Knight (1950) developed resistance against the leaf curl disease in *G. barbadense* by repeated cycles of selection and, from the nature of response to selection, it was inferred that resistance to leaf curl was controlled by minor genes. Tarr (1951) was of the opinion that resistance against the virus in *G. barbadense* may not always be a stable quality. He reported that no major gene was involved in conferring resistance to the disease, and he suggested that resistance may be due to the cumulative effect of minor genes. On the other hand, Siddig (1968) suggested that the resistance was under the control of a single gene or very closely linked genes.

In addition to the use of natural resistance, it is hoped that, in the future, genetically engineered resistance will be useful for achieving resistance to begomoviruses in cotton and efforts are underway to achieve this objective. The advent of transgenesis offers many ways of obtaining virus resistant plants. It provides the ability to produce crop varieties inherently resistant to pathogen infection. The strategies which have been investigated for their usefulness in providing transgenic resistance against phytopathogenic viruses, including geminiviruses, can be grouped under the terms-pathogen derived resistance (PDR; in which a nucleic acid sequence, which may or may not encode a functional protein, derived from the pathogen is used as the source of resistance) and non pathogen derived resistance (NPDR; in which the source of sequence for resistance is other than the pathogen). Both these strategies have been used to develop transgenic resistance against viruses with a varied level of success. The first report of transgenic resistance against a plant virus involved the expression of the CP of *Tobacco mosaic virus* (Abel *et al*., 1986) and this strategy was subsequently also tried for geminiviruses. Tomato plants expressing the CP of the monopartite begomovirus Tomato yellow leaf curl virus (TYLCV) exhibited delayed symptom development and subsequently showed recovery of symptoms which was dependent on the expression level of the CP (Kunik *et al*., 1994).

Resistance using RNAi has also been achieved against geminiviruses by targeting either coding or non-coding regions of the genome. Transient expression of the bipartite begomovirus *Mungbean yellow mosaic virus* (MYMV) IR sequences as an intron spliced hairpin resulted in complete recovery in blackgram plants infected with MYMV (Pooggin *et al.,* 2003). Similarly an intron spliced hairpin construct containing sequences of the IR conserved between the monopartite begomoviruses TYLCV, Tomato yellow leaf curl Sardinia virus (TYLCSV) and *Tomato yellow leaf curl Malaga virus* yielded a broad spectrum resistance when transiently expressed in tomato and *N. benthamiana* plants challenged with these viruses by *Agrobacterium*-mediated inoculation or whitefly transmission. No virus could be detected in plants which were challenged with virus, that had earlier been inoculated with the hairpin construct, using PCR and a positive correlation between resistance and the accumulation of TYLCV-specific siRNAs (the effector of the RNAi response) was observed in silenced plants (Abhary *et al*., 2006).

Similarly various NPDR strategies have been used. For example, dianthin, a potent ribosome inactivating protein isolated from *Dianthus caryophyllus*, has been exploited to engineer transgenic resistance to the bipartite begomovirus African cassava mosaic virus in *N. benthamiana*

(Hong *et al*., 1997). Similarly the RNAse barstar (Zhang *et al*., 2003), an insect symbiont derived virus binding protein (GroEl) (Edelbaum *et al*., 2009) and peptide aptamers (short peptides that interfere with enzyme activity) (Lopez-Ochoa *et al*., 2006) have also shown promise as strategies to obtain resistance against geminiviruses.

Unfortunately there are not many success stories in engineering resistance cotton against begomoviruses. Asad *et al*. (2003), in a proof of concept study, showed that an antisense construct containing partial Rep sequences of CLCuKoV could provide resistance against the virus in tobacco using RNAi. This construct has been transformed into cotton and performs well in small-scale field trials (Shaheen Aftab, personal communication). One limitation of gene silencing based technologies is that they are sequence specific – thus small changes in the targeted virus sequence can overcome the resistance. Thus, it is essential to identify those targets which remain conserved among these viruses. This is no easy task, particularly for CLCuD, where numerous distinct viruses can cause the disease.

SUMMARY

Virus diseases of cotton are an important factor limiting production in some major cotton-growing countries. Whitefly-transmitted viruses are the most important and are currently causing significant losses to cotton production in Pakistan and northwestern India. These viruses are potentially a threat to all cotton-growing areas where the whitefly (*Bemisia tabaci* Gennadius) occurs. Human activity is disseminating both the viruses and their vector to new geographical locations. Exciting progress has been made in understanding the biology of the causal agents of these viral diseases from Asia, Africa and Americas. Cotton-infecting begomoviruses in the Old World are invariably monopartite and are associated with DNA satellites. Two types of DNA satellites, known as alphasatellites and betasatellites, have been identified, although only a betasatellite is essential for symptomatic virus infection of cotton. Cultivated diploid cotton species of Asian/African origin, *Gossypium arboreum* and *G. herbaceum,* are immune to leaf curl disease. Sources of resistance in cultivated tetraploid cotton species (*G. hirsutum* and *G. barbadense*) are limited and emerging virus strains often overcome the available resistance. Recent progress in developing genetically-engineered resistance against begomoviruses is encouraging but commercial exploitation of transgenic cotton varieties will depend on our ability to develop broad-spectrum resistance.

REFERENCES

Abel, P.P., R. Nelson, B. De, N.Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

Abhary, M. K., G.H. Anfoka, M.K. Nakhla, and D.P. Maxwell. 2006. Post-transcriptional gene silencing in controlling viruses of the Tomato yellow leaf curl virus complex. Arch. Virol. 151:2349-2363.

Ali, M. 1997. Breeding of cotton varieties for resistance to cotton leaf curl Virus. Pak. J. Phytopathol. 9: 1-7.

Allen, R.M., H. Tucker, and R.A. Nelson. 1960. Leaf crumple disease of cotton in Arizona. Plant Dis. Rep. 44:246-250.

Amin, I., S. Mansoor, L. Amrao, M. Hussain, S. Irum, Y. Zafar, S.E. Bull, and R.W. Briddon. 2006. Mobilisation into cotton and spread of a recombinant cotton leaf curl disease satellite. Arch. Virol. 151:2055-2065.

Amrao, L., S. Akhter, M.N. Tahir, I. Amin, R.W. Briddon, and S. Mansoor. 2010a. Cotton leaf curl disease in Sindh province of Pakistan is associated with recombinant begomovirus components. Virus Res. 153:161-165.

Amrao, L., I. Amin, S. Shahid, R.W. Briddon, and S. Mansoor. 2010b. Cotton leaf curl disease in resistant cotton is associated with a single begomovirus that lacks an intact transcriptional activator protein. Virus Res. 152:153-163.

Asad, S., W.A.A. Haris, A. Bashir, Y. Zafar, K.A. Malik, M.N. Malik, and CP. Lichtenstein, 2003. Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. Arch. Virol. 148:2341-2352.

Azhar, M. T., I. Amin, Z.I. Anjum, M. Arshad, R.W. Briddon, and S. Mansoor. 2010. Both malvaceous and non-malvaceous betasatellites are associated with two wild cotton species grown under field conditions in Pakistan. Virus Genes 41:417-424.

Bedford, I.D., R.W. Briddon, J.K. Brown, R.C. Rosell, and P.G. Markham. 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Ann. Appl. Biol. 125:311-325.

Briddon, R.W., J.K. Brown, E. Moriones, J. Stanley, M. Zerbini, X. Zhou, and C.M. Fauquet. 2008. Recommendations for the classification and nomenclature of the DNA-β satellites of begomoviruses. Arch. Virol. 153:763-781.

Briddon, R.W., S.E. Bull, I. Amin, A.M. Idris, S. Mansoor, I.D. Bedford, P. Dhawan, N. Rishi, S.S. Siwatch, A.M. Abdel-Salam, J.K. Brown, Y. Zafar, and P.G. Markham. 2003. Diversity of DNA β: a satellite molecule associated with some monopartite begomoviruses. Virology 312:106-121.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner, and P.G. Markham. 2000. Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. Virus Genes 20:17-24.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner, K. Saunders, J. Stanley, Y. Zafar, K.A. Malik, and P.G Markham. 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology 285:234-243.

Briddon, R.W. and P.G. Markham. 2001. Cotton leaf curl virus disease. Virus Res. 71:151-159.

Brown, J.K. 1997. The biology and molecular epidemiology of the geminiviridae subgroup III. pp. 125-195. *In:* G. Stacey and N.T. Keen (eds.). Plant-Microbe Interactions. Chapman and Hall, N.Y.

Brown, J.K. and J. Bird. 1992. Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean basin. Plant Dis. 76:220-225.

Brown, J.K., S.A. Coats, I.D. Bedford, P.G. Markham, J. Bird, and D.R. Frohlich. 1995. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera:Aleyrodidae). Biochem. Genet. 33:205-214.

Brown, J.K., J.D. Mihail, and M.R. Nelson. 1987. Effects of cotton leaf crumple virus on cotton inoculated at different growth stages. Plant Dis. 71:699-703.

Brown, J.K. and M.R. Nelson. 1984. Geminate particles associated with cotton leaf crumple disease in Arizona. Phytopathology 74:987-990.

Brown, J.K. and M.R. Nelson. 1987. Host range and vector relationships of cotton leaf crumple virus. Plant Dis. 71:522-524.

Bull, S.E., W.S. Tsai, R.W. Briddon, P.G. Markham, J. Stanley, and S.K. Green. 2004. Diversity of begomovirus DNA β satellites of non-malvaceous plants in east and south east Asia. Arch. Virol. 149:1193 - 1200.

Chen, L.F., M. Rojas, T. Kon, K. Gamby, B. Xoconostle-Cázares, and R.L. Gilbertson. 2009. A severe symptom phenotype in tomato in Mali is caused by a reassortant between a novel recombinant begomovirus (Tomato yellow leaf curl Mali virus) and abetasatellite. Mol. Plant Pathol. 10:415-430.

Cheng, X., X. Wang, J. Wu, R.W. Briddon, and X. Zhou. 2011. βC1 encoded by tomato yellow leaf curl China betasatellite forms multimeric complexes *in vitro* and *in vivo*. Virology 409:156-62.

Corrêa, R.L., T.F. Silva, J.L. Simões-Araújo, P.A.V. Barroso, M.S. Vidal, and M.F.S. Vaslin, 2005. Molecular characterization of a virus from the family Luteoviridae associated with cotton blue disease. Arch. Virol. 150:1357-1367.

Cui, X., G. Li, D. Wang, D. Hu, and X. Zhou. 2005. A begomovirus DNAβ-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. J. Virol. 79:10764-10775.

Das, S., A. Roy, R. Ghosh, S. Paul, S. Acharyya, and S. Ghosh. 2008. Sequence variability and phylogenetic relationship of betasatellite isolates associated with yellow vein mosaic disease of mesta in India. Virus Genes. 37: 414-424.

Dickson, R.C., M.M. Johnson, and E.F. Laird. 1954. Leaf crumple, a virus disease of cotton. Phytopathology 44:479-480.

Distéfano, A., I.B. Kresic, and H. Hopp. 2010. The complete genome sequence of a virus associated with cotton blue disease, cotton leafroll dwarf virus, confirms that it is a new member of the genus *Polerovirus*. Arch. Virol. 155:1849-1854.

Dry, I., L.R. Krake, J.E. Rigden, and M.A. Rezaian. 1997. A novel subviral agent associated with a geminivirus: the first report of a DNA satellite. Proc. Natl. Acad. Sci. USA 94:7088-7093.

Edelbaum, D., R. Gorovits, S. Sasaki, M. Ikegami, and H. Czosnek. 2009. Expressing a whitefly GroEL protein in *Nicotiana benthamiana* plants confers tolerance to tomato yellow leaf curl virus and cucumber mosaic virus, but not to grapevine virus A or tobacco mosaic virus. Arch. Virol. 154:399-407.

Eini, O., S. Dogra, L.A. Selth, I.B. Dry, J.W. Randles, and M.A. Rezaian. 2009. Interaction with a host ubiquitin-conjugating enzyme is required for the pathogenicity of a geminiviral DNA β satellite. Mol. Plant Microbe In. 22:737-746.

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M.,Zerbini, and X. Zhou. 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol. 153:783-821.

Frischmuth, T., M. Engel, and H. Jeske. 1997. Beet curly top virus DI DNA-mediated resistance is linked to its size. Mol. Breeding 3:213-217.

Frischmuth, T., M. Ringel, and C. Kocher. 2001. The size of encapsidated single-stranded DNA determines multiplicity of African cassava mosaic virus particles. J. Gen. Virol. 82:673-676.

Frischmuth, T. and J. Stanley. 1991. African cassava mosaic virus DI DNA interferes with the replication of both genomic components. Virology 183:539-544.

Gronenborn, B. 2004. Nanoviruses: genome organisation and protein function. Vet. Microbiol. 98:103-110.

Harrison, B.D., Y.L. Liu, S. Khalid, S. Hameed, G.W. Otim-Nape, and D.J. Robinson. 1997. Detection and relationships of cotton leaf curl virus and allied whitefly-transmitted geminiviruses occurring in Pakistan. Ann. Appl. Biol. 130:61-75.

Hong, Y., K. Saunders, and J. Stanley. 1997. Transactivation of dianthin transgene expression by African cassava mosaic virus AC2. Virology 228:383-37.

Hu, C.C., Y.H. Hsu, and N.S. Lin. 2009. Satellite RNAs and satellite viruses of plants. Viruses 1:1325-1350.

Hussain, T. and Ali, M. 1975. A review of cotton diseases of Pakistan. Pakistan Cotton 19:71-86.

Hussain, T. and T. Mahmood. 1988. A note on leaf curl disease of cotton. Pakistan Cotton 32:248-251.

Hutchinson, J.B., and R.L. Knight. 1950. Response of cotton to leaf curl disease. J. Genet. 50:100-111.

Hussain, M., S. Iram, S. Mansoor, and R.W. Briddon. 2009. A single species of betasatellite is prevalent in chili across north central Pakistan and shows phylogeographic segregation. J. Phytopathol. 157:576-579.

Idris, A.M., R.W. Briddon, S.E. Bull, and J.K. Brown. 2005. Cotton leaf curl Gezira virus-satellite DNAs represent a divergent, geographically isolated Nile Basin lineage: predictive identification of a satDNA REP-binding motif. Virus Res. 109:19-32.

Idris, A.M. and J.K. Brown. 2002. Molecular analysis of cotton leaf curl virus-Sudan reveals an evolutionary history of recombination. Virus Genes 24:249-256.

Idris, A.M. and J.K. Brown. 2004. Cotton leaf crumple virus is a distinct Western Hemisphere begomovirus species with complex evolutionary relationships indicative of recombination and reassortment. Phytopathology 94:1068-1074.

Jose, J. and R. Usha. 2003. Bhendi yellow vein mosaic disease in India is caused by association of a DNA β satellite with a begomovirus. Virology 305:310–317.

Kirkpatrick, T.L., and C.S. Rothrock (eds.). 2001. Compendium of cotton diseases. 2 ed. APS Compendium of Plant Disease Series. St. Paul, Minn.

Kirkpatrick, T.W. 1931. Further studies on leaf-curl of cotton in Sudan. B. Entomol. Res. 22:323-363.

Kirthi, N., C.G.P. Priyadarshini, P. Sharma, S. P. Maiya, V. Hemalatha, P. Sivaraman, P. Dhawan, N. Rishi, and H.S. Savithri. 2004. Genetic variability of begomoviruses associated with cotton leaf curl disease originating from India. Arch. Virol. 149:2047-2057.

Kon, T., M.R. Rojas, I.K. Abdourhamane, and R.L. Gilbertson. 2009. The role and interactions of begomoviruses and satellite DNAs associated with okra leaf curl disease in Mali, West Africa. J. Gen. Virol. 90:1001-1013.

Kumar, A., J. Kumar, and J. Khan. 2010. Sequence characterization of cotton leaf curl virus from Rajasthan: phylogenetic relationship with other members of geminiviruses and detection of recombination. Virus Genes 41:432-40.

Kumar, P.P., R. Usha, A. Zrachya, Y. Levy, H. Spanov, and Y. Gafni. 2006. Protein-protein interactions and nuclear trafficking of coat protein and bC1 protein associated with Bhendi yellow vein mosaic disease. Virus Res. 122:127-136.

Kunik, T., R. Salomon, D. Zamir, N. Navot, M. Zeidan, I. Michelson, Y. Gafni, and H. Czosnek. 1994. Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. Biotechnol. 12:500-504.

Lopez-Ochoa, L., J. Ramirez-Prado, and L. Hanley-Bowdoin. 2006. Peptide aptamers that bind to a geminivirus replication protein interfere with viral replication in plant cells. J. Virol. 80:5841-5853.

Mahmood, T. 1999. Cotton leaf curl disease and its status in Pakistan. Proc. ICAC-CCRI Regional consultation on insecticide resistance management in cotton, CCRI, Multan, Pakistan 28th June to 1st July, 1999, pp:234-244.

Mansoor, S., I. Amin, S. Iram, M. Hussain, Y. Zafar, K.A. Malik, and R.W. Briddon. 2003a. Breakdown of resistance in cotton to cotton leaf curl disease in Pakistan. Plant Pathol. 52:784.

Mansoor, S., I. Bedford, M.S. Pinner, J. Stanley, and P.G. Markham. 1993. A whitefly-transmitted geminivirus associated with cotton leaf curl disease in Pakistan. Pak. J. Bot. 25:105-107.

Mansoor, S., R.W. Briddon, S.E. Bull, I.D. Bedford, A. Bashir, M. Hussain, M. Saeed, M.Y. Zafar, K.A. Malik, C. Fauquet, and P.G. Markham. 2003b. Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA β. Arch. Virol. 148:1969-1986.

Mansoor, S., S.H. Khan, A. Bashir, M. Saeed, Y. Zafar, K.A. Malik, R.W. Briddon, J. Stanley, and P.G. Markham. 1999. Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. Virology 259:190-199.

Maruthi, M. N., J. Colvin, S. Seal, G. Gibson, and J. Cooper. 2002. Co-adaptation between cassava mosaic geminiviruses and their local vector populations. Virus Res. 86:71-85.

Mubin, M., M.S. Shahid, M.N. Tahir, R.W. Briddon, and S. Mansoor. 2010. Characterization of begomovirus components from a weed suggests that begomoviruses may associate with multiple distinct DNA satellites. Virus Genes 40:452-457.

Murant, A F. and M.A. Mayo. 1982. Satellites of plant viruses. Ann. Rev. Phytopathol. 20:49-70.

Nadeem, A., Z. Weng, M.R. Nelson, and Z. Xiong. 1997. Cotton leaf crumple virus and cotton leaf curl virus are distantly related geminiviruses. Mol. Plant Pathol. http://www.bspp.org.uk/mppol.1997/0612nadeem

Nawaz-ul-Rehman, M.S., N. Nahid, S. Mansoor, R.W. Briddon, and C.M. Fauquet. 2010. Post-transcriptional gene silencing suppressor activity of the alpha-Rep of non-pathogenic alphasatellites associated with begomoviruses. Virology 405:300-308.

Paul, S., A. Roy, R. Ghosh, S. Das, S. Chaudhuri, and S.K. Ghosh. 2008. Molecular characterisation and sequence variability of betasatellites associated with leaf curl disease Kenaf (*Hibiscus canabinus* L.) from different geographic locations of India. Acta Virol. 52:251-256.

Pooggin, M., P.V. Shivaprasad, K. Veluthambi, and T. Hohn. 2003. RNAi targeting of DNA virus in plants. Nat. Biotechnol. 21:131-132.

Qazi, J., I. Amin, S. Mansoor, J. Iqbal, and R.W. Briddon. 2007. Contribution of the satellite encoded gene βC1 to cotton leaf curl disease symptoms. Virus Res. 128:135-139.

Rahman, M., D. Hussain, T.A. Malik, and Y. Zafar. 2005. Genetics of resistance against cotton leaf curl disease in *Gossypium hirsutum*. Plant Pathol. 54:764-772.

Rahman, M., D. Hussain, and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton cultivars–genotypes by DNA fingerprinting technology. Crop Sci. 42:2137-2144.

Rojas, M.R., A.M. Noueiry, W.J. Lucas, and R.L. Gilbertson. 1998. Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form- and size-specific manner. Cell 95:105-113.

Roy, A., S. Acharyya, S. Das, R. Ghosh, S. Paul, R.K. Srivastava, and S.K. Ghosh. 2009. Distribution, epidemiology and molecular variability of the begomovirus complexes associated with yellow vein mosaic disease of mesta in India. Virus Res. 141:237-246.

Saeed, M., S.A.A. Behjatnia, S. Mansoor, Y. Zafar, S. Hasnain, and M.A. Rezaian. 2005. A single complementary-sense transcript of a geminiviral DNA β satellite is determinant of pathogenicity. Mol. Plant Microbe In. 18:7-14.

Saeed, S., Y. Zafar, J.W., Randles, and M.A. Rezaian. 2007. A monopartite begomovirus-associated DNA β satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection. J. Gen. Virol. 88:2881-2889.

Saunders, K., I.D. Bedford, R.W. Briddon, P.G. Markham, S.M. Wong, and J. Stanley. 2000. A unique virus complex causes Ageratum yellow vein disease. Proc. Natl. Acad. Sci. USA 97:6890-6895.

Saunders, K., I.D. Bedford, and J. Stanley. 2002. Adaptation from whitefly to leafhopper transmission of an autonomously-replicating nanovirus-like DNA component associated with ageratum yellow vein disease. J. Gen. Virol. 83:909-915.

Saunders, K., A. Norman, S. Gucciardo, and J. Stanley. 2004. The DNA β satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (βC1). Virology 324:37-47.

Saunders, K. and J. Stanley. 1999. A nanovirus-like component associated with yellow vein disease of *Ageratum conyzoides*: evidence for interfamilial recombination between plant DNA viruses. Virology 264:142-152.

Seal, S.E., F. van den Bosch, and M.J. Jeger. 2006. Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Crit. Rev. Plant Sci. 25:23-46.

Shahid, M.S., S. Mansoor, and R.W. Briddon. 2007. Complete nucleotide sequences of cotton leaf curl Rajasthan virus and its associated DNA β molecule infecting tomato. Arch. Virol. 152:2131-2134.

Siddig, M.A. 1968. Genetics of resistance to cotton leaf curl in Sakel cotton. J. Agric. Sci. Conf. 70:99-103.

Silva, T.F., R.L. Corrêa, Y. Castilho, P. Silvie, J.L. Bélot, and M.F.S. Vaslin. 2008. Widespread distribution and a new recombinant species of Brazilian virus associated with cotton blue disease. Virol. J. 5:123.

Singh, J., Sohi, A. S., and Mann, H. S. 1997. Screening of cotton germplasm against cotton leaf curl viral disease using its vector *Bemisia tabaci* (Genn.). J. Res. Punjab Agricultural University 34:294-298.

Sivalingam, P., V. Malathi, and A. Varma. 2010. Molecular diversity of the DNA-β satellites associated with tomato leaf curl disease in India. Arch. Virol. 155:757-764.

Stanley, J., D.M. Bisaro, R.W. Briddon, J.K. Brown, C.M. Fauquet, B.D. Harrison, E.P. Rybicki, and D.C. Stenger. 2005. Geminiviridae. pp. 301-326. *In*: Virus Taxonomy, VIIIth Report of the ICTV. C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, and L.A. Ball (Eds.), Elsevier/Academic Press, London, UK.

Tarr, S.A.J. 1951. Leaf curl diseases of cotton. Commonwealth Mycological Institute, Kew, Surrey, UK.

Timchenko, T., F. de Kouchkovsky, L. Katul, D. David, H.J. Vetten, and D.B. Gronenborn. 1999. A single Rep protein initiates replication of multiple genome components of Faba bean necrotic yellows virus, a single-stranded DNA virus of plants. J. Virol. 73:10173–10182.

Timchenko, T., L. Katul, Y. Sano, F. de Kouchkovsky, H.J. Vetten, and B. Gronenborn. 2000. The master Rep concept in nanovirus replication: identification of missing genome components and potential for natural genetic reassortment. Virology 274:189-195.

van Schaik, P.H., D.C. Erwin, and M. Garber. 1962. Effects of time on symptom expression of the leaf-crumple virus on yield and quality of fiber cotton. Crop Sci. 2:275-277.

Voinnet, O. 2001. RNA silencing as a plant immune system against viruses. Trends Genet. 17:449-459.

Voinnet, O., Y.M. Pinto, and D.C. Baulcombe. 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. P. Natl. Acad. Sci. USA 23:14147-14152.

Watkins, G.M. 1981. Compendium of cotton diseases. American Phytopathological Society. ISBN-10:0890540314

Wilson, F.D. and J.K. Brown. 1991. Inheritance of resistance to cotton leaf crumple virus in cotton. J. Hered. 80:508-509.

Wu, P.J. and X.P. Zhou. 2005. Interaction between a nanovirus-like component and the Tobacco curly shoot virus/satellite complex. *Acta Bioch Bioph Sin* 37:25-31.

Yang, J.Y., M. Iwasaki, C. Machida, Y. Machida, X. Zhou, and N.H. Chua. 2008. βC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Gene. Dev. 22:2564-2577.

Zhang, P., J. Fütterer, P. Frey, I. Potrykus, J. Puonti-Kaerlas, and W. Gruissem. 2003. Engineering virus-induced ACMV resistance by mimicking a hypersensitive reaction in transgenic cassava plants. pp. 143–146. *In*: I.K. Vasil (ed.). Plant Biotechnology 2002 and Beyond Norwell and Dordrecht: Kluwer Academic.

Zhou, X., Y. Liu, D.J. Robinson, and B.D. Harrison. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. J. Gen. Virol. 79:915-923.