

# Molecular Diagnostics and Management Strategies for Begomovirus-Induced Cotton Leaf Curl Disease: The Role of Weeds as Reservoirs and Vectors

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## ABSTRACT

Begomoviruses are known to cause Cotton Leaf Curl Disease (CLCuD), and this disease is devastating to the production of cotton especially in South Asia, Africa as well as the Middle East. The virus is transmitted by *Bemisia tabaci* (whitefly) but the weeds serve as reservoirs and vectors that sustain the virus even between cotton-growing seasons. Conventional diagnostic tests such as PCR and ELISA are viable and time consuming and also need specialized equipments, limiting them to remote locations. CRISPR-based technology and Next-Generation Sequencing (NGS) provide a more effective and quicker method of detection. A major approach to the management of CLCuD is Integrated Pest Management (IPM), weed management, biological management, and development of genetically resistant cotton varieties. The review is based on the concept of molecular diagnostics, the importance of weeds in the spread of the virus, and the existing control strategies, providing the facts on sustainable ways to manage the spread of CLCuD.

**Keywords:** Begomovirus, Cotton Leaf Curl Disease, weeds, CRISPR, Next-Generation Sequencing (NGS)

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## Introduction

Begomoviruses (family; *Geminiviridae*) are some of the most impactful plant viruses to crops in the tropical and subtropical areas. Their circular single-stranded DNA genomes (2.5-5.2 kb all geminiviruses) are packaged in typical geminate particles, and the begomoviruses are mostly spread by whiteflies as part of the *Bemisia tabaci* species complex (1-2). Begomovirus epidemics are maintained in the field due to the wide host range, the high degree of genetic diversification (including recombination), and the prevalence of mixed infections (3). The outcomes of diseases in numerous Old World pathosystems are also predetermined by parallel circular ssDNA satellites (alpha-, beta- and delta-satellites), which may regulate the severity of the symptoms and determine the epidemiological processes (4).

Cotton leaf curl disease (CLCuD) can be used as an example on how such evolutionary plasticity can disrupt the production systems of fibers (Brown and Khan, 2022). The first case of CLCuD was documented in 1912 in Nigeria and then went on to cause a significant series of outbreaks in cotton-producing areas of the Indian subcontinent (4-5). Association with satellites, in particular, cotton leaf curl Multan betasatellite, which is frequently necessary to express the symptoms powerfully and may sometimes exacerbate the disease severity in association with the begomoviruses that cause the disease, leads to the disease (4). Common symptoms are; curling of leaves, thickening of veins, enations in the underside of leaves, stunting and terrible yield penalties. Controlling vectors over a long period is hard since it is not easy to manage vectors due to their size and the evolution of viruses in response to hosts, which occasionally develop resistance-breaking variants such as the Burewala strain (5).

These challenges are also enhanced by weeds and other non-crop hosts that provide an epidemiology bridge between cycles of cropping, which sustain the population of whiteflies and allow cross-host transmission of viruses and satellites. As an example, field-edge weed *Digera arvensis* in Pakistan has been identified to be a reservoir of agriculturally significant begomoviruses alongside alpha- and betasatellites indicating the weeds as primary inoculum reservoirs in surrounding fields (6).

Since early warning and proper strain monitoring are the key to successful intervention, molecular diagnostics play a central role in CLCuD surveillance and management. Traditional PCR continues to be a standard backbone technology which is backed up by highly reactive degenerate and general primer sets that have been designed against geminiviruses (7-8). Most recently, CRISPR-based nucleic-acid detector-based platforms (e.g., Cas12/Cas13-assays with an isothermal amplification) are adapted to identify plant viruses fast, sensitively, and possibly even in the field and replace laboratory systems based on PCR and sequencing (9). The review plays a critical role in learning the Begomoviruses in Cotton Leaf Curl Disease (CLCuD) and how to close the gaps in the diagnostic tools as well as in management approaches, which are sustainable.

## Begomovirus and Cotton Leaf Curl Disease

The cause of Cotton Leaf Curl Disease (CLCuD) is begomoviruses, single-stranded DNA (ssDNA) viruses, which belong to the family *Geminiviridae* (10-11). Their genomes tend to be between 2.5 and 3 kilobases, and consequently, quite small yet powerful in the affliction of plants (12). *Bemisia tabaci* (whitefly) propagand is also an important agent in Begomovirus transmission. The whitefly becomes infected when it fed on the phloem of the plants which are sick and then infects the non-sick plants as they feed (13). The virus genome harbors the key proteins like coat protein (CP) and replication-related protein (Rep), which are necessary to finish the process of replication and pathogenicity of the virus in cotton plants (14-15).

Curling of cotton leaves, yellowing and stunting of cotton plants are the main symptoms of CLCuD (16). The symptoms result in a significant decrease in boll development and the yield in general which has a great impact on crop productivity. In severe cases, affected plants can die before development of bolls and total crop loss is experienced (17). The cost of CLCuD to the economy is overwhelming and losses in yield are reported to have reached up to 70 per cent in the area where the level of infection is high. Begomoviruses are quite challenging to manage, due to the seasonality of the disease and the nature of their transmission, with areas with large populations of whiteflies and heavy weed captures that research the virus between cotton-growing seasons making it especially difficult to contain it (12).

**Table 2: Key Characteristics of Begomoviruses and Cotton Leaf Curl Disease**

Characteristic	Details
<b>Virus Family</b>	<i>Geminiviridae</i>
<b>Genus</b>	<i>Begomovirus</i>
<b>Genome</b>	Single-stranded DNA (ssDNA) genome, typically 2.5-3 kilobases
<b>Primary Vectors</b>	Whitefly ( <i>Bemisia tabaci</i> )
<b>Major Viruses Involved</b>	<i>Cotton Leaf Curl Kokhran Virus</i> (CLCuKoV), <i>Cotton Leaf Curl Multan Virus</i> (CLCuMV)
<b>Transmission Method</b>	Whiteflies acquire the virus while feeding on infected plants and transmit it to healthy plants. Weeds act as virus reservoirs between cropping cycles.
<b>Symptoms</b>	Leaf curling, yellowing, stunting, enations (swelling on the underside of leaves), reduced boll formation, and yield loss.
<b>Impact on Cotton</b>	Yield loss up to 70% in heavily infected areas; total crop loss in extreme cases.
<b>Economic Impact</b>	Significant economic losses in cotton-producing regions, particularly in South Asia and Africa.
<b>Control Strategies</b>	Integrated Pest Management (IPM), weed management, use of resistant cotton varieties, molecular diagnostics, and biological control of whiteflies.

## Weeds as Vectors and Reservoirs of Begomoviruses

Weeds are also critical in Begomovirus transmission since they act as asymptomatic vectors of the virus (18). These viruses have several species

of weeds that serve as the source of continuous infection to the whiteflies (Figure 1) that are infected in the course of feeding. The reservoirs commonly used as weed include *Chenopodium ambrosioides*, *Cucumis sativus*, and *Abutment indicum* (19). These are weeds that carry the virus throughout the off-season and help in causing the disease in the next cotton-growing season.

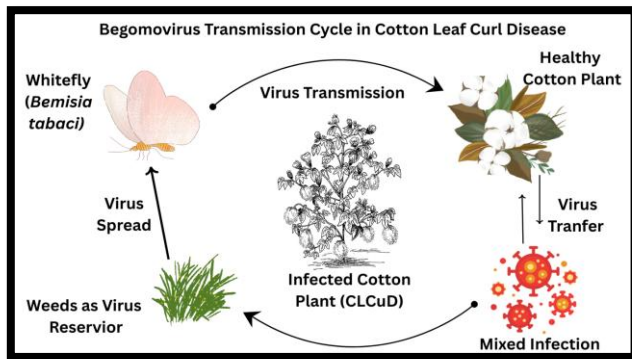


Figure 1: Begomovirus Transmission Cycle in CLCuD.

Weeds are reservoirs and vectors (Figure 1), which is very critical in spreading the virus. Whiteflies that feed on infected weeds also obtain the Begomoviruses which are then transmitted to cotton plants. This is a recurring cycle transmission process which keeps the virus in the ecosystem and causes it to spread constantly without necessarily relying on the presence of infected cotton crops (20). Moreover, the virus can be spread through mechanical routes as well agricultural practices (weeding or physical harm) (13).

Various ecological elements such as temperature, rainfall and abundance of weeds affect the transmission of Begomoviruses. On the monsoon season, the weeds are more and this gives the virus more hosts. However, the dry season can cause an increase in the concentration of virus particles of the weed because of a decrease in dilution and an increase in the risk of transmission by whiteflies (12).

#### Molecular Diagnostics for Begomovirus and CLCuD

Traditionally, the identification of Begomoviruses in Cotton Leaf Curl Disease (CLCuD) was based on Polymerase Chain Reaction (PCR), as well as Enzyme-Linked Immunosorbent Assay (ELISA). PCR is very sensitive that it the viral loads can be detected by amplification of viral DNA sequence specific to the virus and can discriminate between virus strain and mixed infection (14). ELISA identifies viral proteins and is applied in field surveys, but it has several limitations because it is time-consuming and needs special equipment, which prevents its application in remote settings or during rapid outbreaks (12-18).

Recent developments of CRISPR-based technologies and Next-Generation Sequencing (NGS) have enhanced the method of detecting viruses. CRISPR/Cas systems have high sensitivity and speed, enabling high speed and detection of Cas12a and Cas13a, and are suitable on-site detection, best suited in the field (21). NGS enables the comprehensive sequencing of viral genomes to monitor the evolutionary process of viruses and enables the detection of more virulent viruses, although it involves advanced equipment, which restricts its application in the field of standard diagnostics (18).

Portable diagnostic kits based on Loop-Mediated Isothermal Amplification (LAMP) and subsequent lateral flow assays will be developed to increase their accessibility. These are affordable, fast, and suitable in real-time detection, on site, in remote areas (18).

#### Management Strategies for Begomovirus and CLCuD

##### Integrated Pest Management (IPM)

Integrated Pest Management (IPM) is an integrated method that incorporates biological, cultural, and chemical control to control the population of pests and control the spread of Begomoviruses. The biological control is relevant in the control of the population of whitefly that comprises the principal vectors of Begomoviruses. The natural predators such as predator mites and ladybugs can be used to reduce the presence of the whiteflies and control the spread of the virus (17). It is environmental friendly and the application of chemical insecticides is reduced. The cultural control methods also go along with the biological control. The methods of controlling the whitefly also involve the crop rotation whereby the cotton is interchanged with other crops and such a method disrupts the life cycle of the whitefly and reduces the population of the same. A second important cultural control activity is eradication of the infected plants that will enable prevention of the further development of the virus in the cotton fields. Moreover, the effect of CLCuD is it can be controlled with the help of

resistant kinds of cotton varieties. These sorts are genetically engineered to resist viral diseases and reduce sales loss (22).

Although biological and cultural controls are very important, there is a possibility that chemical control is required in extreme cases of infestations. Chemical insecticides can be used to manage the number of whiteflies, but taking into account the fact that excessive use of insecticides may cause resistance, it is crucial to use and apply them as a component of an integrated pest management system (22).

##### Weed Management

The presence of weeds is also important to the Begomovirus reservoir as the virus could survive in between cotton-growing seasons in weeds. It is thus necessary to manage the spread of the virus through effective management of the weed. One of them is the use of herbicides, which is used to kill weeds, thereby decreasing the number of reservoirs that can be inhabited by the virus, which is transmitted by whiteflies. Nevertheless, the use of herbicides should be regulated to prevent the emergence of resistant species of weeds (12). Regular weeding of cotton field is also very effective way of limiting viral transmission. The risk of extending the virus is also reduced since the weeds are eliminated and they are the carriers of the virus, particularly when cotton is not under cultivation.

##### Genetic Resistance in Cotton

Creation of genetically resistant varieties of cotton is one of the remedies in the long-term to the CLCuD management. The varieties have been made resistant to the virus and its vectors and have broad protection against CLCuD. Several resistant types are already reported and there are more developments that are underway to improve their resistance against progressive strains of Begomoviruses. This kind of genetic-based solution coupled with other control measures provides a long-term solution to the reduction of the impact of CLCuD on cotton production (20).

##### Conclusion

Begomoviruses and Cotton Leaf Curl Disease (CLCuD) is still a menace to the production of cotton in the world particularly in areas such as South Asia and Africa where the disease is known to incur massive losses in production. Whiteflies (*Bemisia tabaci*) are the major vectors of CLCuD since they contract the virus during feeding on infected plants and then transmit it to other crops that are healthy. Although molecular diagnostics have developed in the form of PCR, CRISPR-based systems, and Next-Generation Sequencing (NGS), which allow one to detect Begomoviruses quickly and accurately, it is hard to control the disease. Conventional diagnostic techniques are effective but tend to be labor intensive and are not applicable in the fields in remote locations.

The weeds serve as reservoirs and vectors of CLCuD, which is a significant factor in the persistence of CLCuD. The viruses are carried by weeds like *Chenopodium ambrosioides* and *Cucumis sativus*, which act as a persistent source of infection and thus weeds are the most important aspect to control Begomoviruses. The combination of the three strategies used in managing CLCuD is integrated management, which entails the use of weed management, biological management (through natural predators of whiteflies), resistant cotton varieties, and modern diagnostics, all of which are necessary in ensuring sustainable management of CLCuD. These integrated measures will go a long way in preventing the proliferation of the disease and provide long term solutions to cotton farmers.

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