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Review Article

Cotton Leaf Curl Virus (CLCuV): An Insight into Disaster

Noor Hassan^{1*}, Mujahid Hussain², Faisal Mushtaq³, Saqib Ali¹ and Muhammad Saqib Shahzad Chaudary⁴

¹Department of Chemistry, Govt. College Township, Lahore, Pakistan

²Department of Biology, University of Florida, Gainesville, United State of America

³Department of Nursing, Sir Ganga Ram Hospital, Lahore, Pakistan

⁴Department of Biology, Govt. College Model Town, Lahore, Pakistan

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*Corresponding Author:

Noor Hassan
Department of Chemistry, Govt. College Township,
Lahore, Pakistan
noorh6784@gmail.com

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ABSTRACT

Disaster of cotton leaf curl virus disease (CLCuVD) is the severe restriction to cotton production wherever it appears, and this dilemma is under discussion by scientist since few decades and still no satisfactory control is obtained. Cotton leaf curl Virus (CLCuV) is a begomovirus belongs to family geminiviridae and transmitted by insect-vector Whitefly complex (*Bemisia tabaci* Gennadius and *Bemisia argentifolii* Bellows & Perring). In Pakistan, disease was first reported by scientist on stray plants in 1967 near Multan, Punjab. In 1992-93 disease epidemic cause yield decline from 9.05 million bales to 8.04 million bales cause severe losses to cotton production in Pakistan. During 1996-97 resistant cotton varieties against CLCuV were introduced but since 2001 new strain Cotton Leaf Curl Burewala Virus (CLCuBV) cause another epidemic by breaking resistance in all available varieties. Excessive use of pesticides to control vector is the serious environmental threat, caused vector resistance, and are carcinogenic and neurotoxic to humans. This review is to cover the history, spread, major losses, Management, Molecular study of relation between host-pathogen and virus resistance strategies to reduce the use of pesticide.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a major fiber crop produced in different regions of globe i.e., South Asia, America, Africa and Australia etc. China is the leading producer of cotton, India and USA are at 2nd and 3rd position respectively, and Pakistan stands at 4th in the list [1]. There are number of biotic and abiotic factors that attack the cotton crop causing quantitative and qualitative loss in Pakistan as well as all over the world. Cotton leaf curl disease (CLCuD) is serious dilemma in different cotton producing regions of the world. CLCuD is caused by a multiple complex of Begomoviruses - having many strains, transmitted by whitefly [2]. CLCuD was first time reported in Nigeria during 1912 [3]. Later the disease was reported from Tanzania and Sudan in 1926 and 1934 respectively. In

Pakistan the disease was first time reported on some stray plant near Multan region in 1967. Both neighbor countries Pakistan and India faced two epidemics, Pakistan faced heavy economic losses during 1992-1997 [4]. During late 1990s resistant varieties developed by conventional breeding methods but in 2001 it was believed that resistance has been broken because that resistant varieties of cotton started showing the symptoms of disease [5]. In 2006-2007 cotton leaf curl Burewala strain broke resistance in all available varieties. So far, there is no resistance against CLCuD in Pakistan and surrounding regions. CLCuV is vector transmitted disease by whitefly (*Bemisia tabaci*) belongs to genus begomovirus and family geminiviridae. This cosmopolitan species cause

devastation by threatening the vigor of host through the fall of cell sap combine with the inoculation of fetid saliva which is coupled with virus transferring. Losses occur due to *B. tabaci*-hauled viruses, through suckling damage, plant physical disarray, honey droplets poisoning and affiliated fungal production [6]. The only possible solution to tackle this disease is to induce genetic resistance in cotton plants.

CLCuD Effect on Yield

Yield loss are related with infectivity time and variety of cotton. CLCuD not only destroy the quantity of yield but also qualitative parameters. Major losses of CLCuD is in early stages but at later stages results minor infection [7].

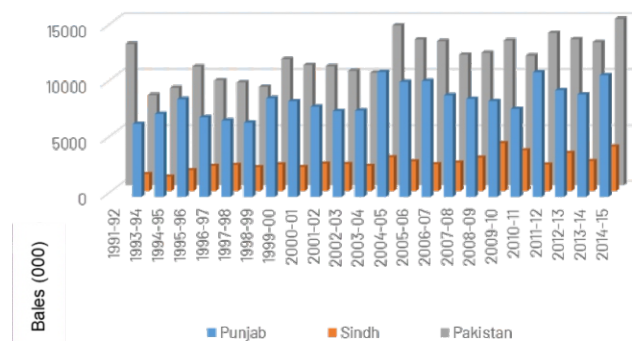


Figure 1: Annual Variation of Cotton Production in Pakistan

Symptoms of Disease

The characteristic symptoms include vein thickening and curling of leaf margins giving a cup shaped appearance. In some cotton varieties formation of small leaf like structure called enation appears [4]. Plants infected during early growth season are stunted and yield is reduced significantly. Mostly, the disease symptoms are induced by DNA satellite known as CLCuLB which is a dominant beta satellite in cotton but another beta satellite known as Chili Leaf Curl beta satellite (ChLCB) is also found associated with the disease [8].

Epidemiology of CLCuD

There are number of environmental factors which affect the epidemiology of CLCuD i.e., rainfall, humidity, temperature and wind. These factors also affect the success or failure of oviposition and egg development of the vector. The disease is directly related to rainfall and humidity with third most influencing factor is temperature [9]. The vector populations increased due to rainfall before seedling as rainfall cause the increase food resources for vector. The overall index of factors affecting is as: rainfall > humidity > vector population > temperature. Rainfall is most influencing factor and temperature is the least [9]. The ailment invasion increased in the highest and lowest temperature range of 33-45 °C and 25-30 °C respectively. Maximum disease incidence at six-week longstanding seedlings and it progressively decreases with rise in

maturity of host [10]. b: represents the species out of total to transmit the disease be *B. tabaci*: additional species.

Vector-Virus Interaction

Begomovirus are the main class of plant viruses spread entirely by *B. tabaci* in an insistent regulative non-productive mode. Insect gut contains some essential protein receptors, which binds with the attachment sites of the virus enabling them to enter in the hemolymph of the insect and midgut protein is the common protein found interacting with Tomato Leaf Curl Disease (ToLCD) and CLCuD [11]. The viruses of both diseases (ToLCD & CLCuD) had not observed replicating in their vector like whitefly but there is the possible transcription of viral genome in whitefly [12]. The intake of virus by *B. tabaci* are carried out to the hemolymph over the epithelial cells of midgut [13]. Virus particles distribute in hemolymph and conducted to salivary glands for spread during the next feeding cycle [14]. The only known begomovirus protein is the coat protein required for insect mediated transmission [15, 16]. Two virus proteins are involved in begomovirus movement; one facilitates the nuclear shuttling and binds to the viral DNA while the other opens the gate of plasmodesmata and promote the transfer of the viral DNA from one cell to another cell [8]. In monopartite Begomoviruses the coat protein (CP/AVI) acts as nuclear shuttling protein (NSP) while the pre coat protein (PCA/AV2) acts as the gate keeper. For bipartite Begomoviruses DNA-B encodes BV1/NSP protein for nuclear shuttling and moment protein (MP/BC1) for plasmodesmatal transfer [17].

Vector	Genus	Genetic makeup	Mode of transmission	No. of species
<i>B. tabaci</i>	<i>Begomovirus</i>	ssRNA	Persistent circulative	288
	<i>Carlavirus</i>	ssRNA	Semipersistent	2 (52) ^b
	<i>Torradovirus</i>	ssRNA	Semipersistent	2 (3) ^c
	<i>Ipomovirus</i>	ssRNA	Semipersistent	6
	<i>Crinivirus</i>	ssRNA	Semipersistent	13

Figure 2: Vector-Virus Interaction

Genetics of CLCuV

CLCuV is a begomovirus belongs to family geminiviridae. The family Geminiviridae consists of large number of Begomoviruses which are circular and single stranded and enclosed in linked icosahedral particles comprising two partial icosahedra (T=1) and about 22x38 nm in size. The family geminiviridae are divided into four genera begomovirus, Mastrevirus, Curtovirus and Topocovirus on the basis of host range, genome organization and insect vector. *Mastrevirus* and *Curtovirus* contain single genomic component and *begomovirus* either one or two. Begomoviruses contain more than 200 species or

members, and is the largest geminivirus genus [18]. Begomoviruses are also divided into New World and Old-World groups on the basis of their topographical source. New World begomoviruses has bipartite genome, and the Old World begomoviruses contain either a monopartite or bipartite genome. Monopartite begomovirus encodes six genes and some components of bipartite (DNA A). The DNA A component of the can replicate independently, but it requires DNA B-encoded BV₁ for nuclear localization and BC₁ for systemic infection in host. Begomoviruses are many and geographically prevalent viruses which cause serious diseases to crops. The beta and alphasatellites genome size is, 50% of the begomovirus. Monopartite begomoviruses depends on both of these satellites [19]. Betasatellites was first time isolated in Australia from tomato plant that was suffering from *Tomato Leaf Curl Virus* (ToLCV) [20]. Betasatellites, previously known as DNA β, are circular single stranded DNAs of approximately 1,350 nucleotides associated with numerous Monopartite begomoviruses which are vital for the introduction of distinctive virus indicators [21]. Betasatellites initiate the disease symptoms, but for spread, encapsidation, transmission and replication it relies on helper viruses. βC₁ genes by betasatellite play vital role in symptoms appearance, suppression of transcriptional and post-transcriptional gene silencing and effect on those genes which are responsive for jasmonic acid. Alphasatellites and betasatellites are not similar to begomoviruses in genome organization, but for the presence of a stem-loop structure that is require for the PCR initiator protein.

Beta satellites association with Begomoviruses

Most begomoviruses associated with betasatellite are characterized as monopartite and belongs to Old World. Betasatellites are vital for introduction of characteristic virus symptoms in host plants. Demonstration by a survey in China shows that TYLCCNV, Malvastrum yellow vein virus (MYVV) and Ageratum yellow vein China virus (AYVCNV), all are associated with betasatellite and depends on it for adequate disease in host and appearance of symptoms [22, 23]. Betasatellite are also host specific in their interaction because betasatellite of Tobacco curly shoot virus enhances disease symptoms in Tobacco but not in Tomato, also a beta satellite related with tomato leaf curl disease in Togo, Africa, had no effect on disease symptoms development in tomato but develop symptoms in other solanaceous crops. Betasatellites have ability of trans-replication by begomoviruses so diversity of begomovirus-betasatellite complex can occur. CLCuD occur in Pakistan is caused by a single betasatellite association, CLCuMuB with six begomovirus species approximately, as a single or multiple infections [2]. In recent time, Cotton leaf curl Gezira virus (CLCuGV) which is associated African CLCuD,

was identified from Southern Pakistan, where CLCuMuB was originate [24]. It is observed that single strand nucleic acid begomoviruses, including Papaya leaf curl China virus (PLCCNV) and Tomato yellow leaf curl Virus (TYLCuV) are able to trans-replicate [25]. This study raises a startling situation in which true monopartite begomoviruses might produce new disease complex by attaining other betasatellites in mix-infected plants. Certainly, serious symptom phenotype in tomato and TYLCuV from Oman are initiated by unique begomovirus-betasatellite complex resulting from re-assortment [26]. Betasatellite association with bipartite begomoviruses is rare and has only reported from India, where betasatellite play role in pathogenesis of bipartite begomovirus was studied in Tomato leaf cur New Delhi virus (ToLCLNDV). Under laboratory condition trans-replication of betasatellite by New World Begomoviruses has been established, but New World begomoviruses association have not been reported, however circular DNA genomics assay shows that betasatellite occur in symptomatic plants in Brazil [27].

Vector Biology

Whiteflies (*B. tabaci*) belongs to order Hemiptera are minute insects commonly described by wax covered wings. Several species of whitefly exist. *B. tabaci* feeds on numerous food, fiber, vegetable and decorative plants, can produce several geneses in a single season. After its first-time observation for 100 years ago, it has become most threatening pest of tropical and sub-tropical climatic zones also in green house production system. It has been reported all over the world except Antarctica because it can accept new environment certainly. Biotype K and H are described from South East Asia. They transmit the virus of family geminiviridae to the wide host range of plants [27]. Life cycle of whitefly consist of eggs, four instars and adult ones. Usually, eggs are elongated to oval shaped but sometime they are bean shaped. The upper end of egg is needle shape and lower end is thick and have pedicel for attachment. Whitefly lay whitish eggs underneath of the leaf. Upto 300 eggs are laid down by the female *B. tabaci* and first instar is known as crawler which have legs for movement. All other instars are sessile and live on the same leaf. Life cycle is temperature and plant species dependent and usually extends upto 20 days at 80 °F. The mode of reproduction of these species is parthenogenesis [28]. *B. tabaci* transmitted viruses which led diseases outbreak and made it global pest, is because of their high reproduction and dispersal rate, insecticides resistance, extreme polyphagy as well as human activities. The major factor in the commencement of disease epidemic is the amplified population of the vector *B. tabaci* which cause the spread of begomovirus within crop and alternate host like weeds. Advantageous climatic conditions, cultivation

of some particular crops or crop varieties, and spread of more fertile B-biotype can enhance the population of *B. tabaci*. Excessive use of pesticide has made easy for *B. tabaci* to select the more resistant strains for reproduction to neutralize the effect of pesticide [29].

Origin of CLCuD Complex

CLCuD become major threat to the cotton as susceptible American Cotton variety S₁₂ was cultivated in Pakistan [4] in 1988. This indicates that the previous grown local varieties had shown certain resistance to the CLCuV. CLCuD was not identified during the first epidemic, but the widespread of CLCuMuV and its inputs for disease with other begomoviruses in Asia fetched scientists' attention to uproot this disease [30]. During 1990s some varieties were yielded by conventional selection and breeding, showing excellent resistance to CLCuMuV. These resistant varieties were cultivated across the Pakistan (Except Sindh) reverted CLCuD to unimportance. Three genes take part in resistance mechanism two for resistance and one for overriding of resistance. However, the mechanism of resistance and nature of genes remained unexplained. Despite almost 10 years efforts then the presence of new strain from 'Burewala', traditional breeding not showed true results to overcome this dilemma as it failed to develop any resistant variety [10]. A few tolerance lines produced acceptable yield showing slight symptoms. However, desired resistant lines against CLCuV was not upcoming. Tolerant lines had problem because they support and spread the inoculum, but better than susceptible ones. Therefore, they act as source for spread of virus [30]. Although, *G. hirsutum* was originated in OW but it appeared as a major fiber crop in NW during 1800s like other cultivated crop species such as tomato and maize. The NW could not fulfil the demand of England during American Civil War which inspired OW colonies to grow *G. hirsutum* [30]. *G. hirsutum* which was higher yielding, replaced the *G. arboreum* (local tolerant cotton) in Indian subcontinent but this NW crops extensively suffered by geminiviruses. The common example is cassava in India and Africa, damaged by *Cassava mosaic virus* (Begomoviruses) and *Maize streak virus* damaging maize in Africa [31].

Resistance to CLCuV

Resistance approaches can cope the problem of cotton leaf curl and promote and save the food fiber and feed for the world. The pest free production of cotton is lifeline for industrial sector [32]. To manage these pests extensive use of chemicals pesticides is performed. These pesticides are carcinogenic and neurotoxic to human. Moreover, chemicals resist in the environment and cause of polluting the land and water also affecting their allies. So, use of resistant varieties is economical and environment friendly approach to tackle the problem. Repeated

chemical use leads to pest resistance again insecticides [33]. To solve the problem of pest resistant and environmental pollution Bt-Cotton has been developed and grown around the world. Successful development of Bt-cotton with *Bacillus thuringiensis* is countless achievement. Transgenic Cotton showed 72-81% resistance against CLCuD as compare to the non-transgenic Cotton [34]. Genetically engineered resistance against viruses in several group of plants have been used on the basis of pathogen derived resistance (PDR). The mechanism of truncated genes expression for resistance to begomoviruses involved in PDR. PDR depends on the approach, the introduction and variation expression in plants viral sequences which obstruct with virus life cycle. The higher efficacy of resistance was shown by virus sequences derived from hairpin double stranded constructs. Moreover, Transcriptional gene silencing (TGS) and post-transcriptional gene silencing, used for successful manipulation of RNA and DNA viruses, affected against Begomoviruses. The RNA interference (RNAi) is an effective tactic to reduce the CLCuD losses [35]. The viral DNA in transgenic plants was small, with no symptoms.

Management of Vector

B. tabaci is the serious insect pest to the agriculture and attacks over 500 species of plants through-out the world. *B. tabaci* is an important pest and damage crops through sucking phloem sap, transfer more than 200 virus species and accounts for 100% loss. In past decades, chemical pesticides were the only strategy to control this vector. Chemical pesticides cause environmental pollution and different allergic diseases in humans and also enhancing resistance in *B. tabaci* [36]. Because of this reason Integrated Pest Management (IPM) is a common practice to control the pest attack. Field sanitation, cultural, physical, chemical control and genetic resistance are the components of IPM. Genetic resistance has vital contribution to control the insect *B. tabaci* [37]. Minimum use of insecticides makes biological control effective. Therefore, technologies which can decrease the insect population on crops in eco-friendly approach and provide longstanding efficacy are the ultimatum to scientists and society.

Essential Oils

Insect behavior on host is controlled by the physical, biological, visual and chemical stimuli. Plant stimuli may hinder or favor the feeding on host. Some chemical stimuli which hinder insect activity are essential oils produced by plants as secondary metabolites. These essential oils include fatty acids, terpenoids, phenols, fatty acids alcohols and aldehydes. They act directly or indirectly as antifeedant compounds or toxins and can be effective for the control of *B. tabaci* [38]. For example, *Paphiopedilum*

callosum and *Piper hispidinervum* produce more than 20 compounds as essential oils mainly phenylpropanoid safrole. Terpenes and volatile phenylpropanoid from different plants are capable of insect mortality. Safrole has antifeedant and growth hindering activity for *Tribolium castoreum*[39].

RNA Silencing

RNA silencing or RNA interference (RNAi) is a phenomenon of defense against plant pathogen which especially has genome i.e., viruses and transposons. The significance of RNA silencing in plants is noticeable from the fact that viruses also have started to produce suppressor to overcome the effect of gene silencing [40]. The mechanism of gene silencing is consisting of two phases i.e. the initiation phase and effector phase. In initiation phase the Dicer enzyme cut the double stranded introduced small interfering RNA (siRNA) or microRNA (mRNA) into 21-23 bp dsRNA. The dsRNA has two strands i.e. guide strand and passenger strand. In effector phase, the guide strand of dsRNA along with the Dicer joint with RNA induced silencing complex (RISC) which is multi-protein complex. However, the passenger strand is degraded by a certain Argonaute proteins [41]. The guide strands lead the whole RISC toward the viral genome or mRNA and cause the integration of viral genome. RNA silencing has proven helpful in interfering many Begomoviruses[42].

Biocontrol

Frequent use of pesticides against vector *B. tabaci* resulted in the development of resistant strains against pesticides. Moreover, pesticides pose threats to human and environment, this approach led to alternative mechanism of controlling the pest. There are chances of starving 100 million people by the end of 2030 due to effects of climate change. Bio control agents are less harmful to human and environment, offers affective substitute of chemicals[43]. Entomopathogenic fungus (EPF) are one of best natural enemy of insects and pests. Currently, about 700 species from 90 genera of pathogenic fungus have been described. *Beauveria spp.*, *Metarhizium spp.*, *Lecanicillium spp.* And *Isaria spp.*, are used as mycoinsecticides around the world [44], from which *Beauveria bassiana* contain *Naturalis* and *Lecanicillium* contain *Mycotal* products which have high potential against *B. tabaci* Q biotype. However environmental factors are the major problem in EPF control under field conditions, the progress, sporulation, infection and existence are related with humidity, free moisture, temperature and radiations[45].

Chemicals

There is a list of commercially available chemical approved in the market for the control of vector (Whitefly). However chemical pose serious threat to environment and human

being as well. Moreover, extensive use of agrochemicals is leading to the climate change around the world and raising the temperature.

CONCLUSIONS

Cotton is an important crop of Pakistan for commercial point of view and its production has server affected by the Cotton leaf curl virus. The farmers have stopped planting cotton due to the massive losses. The best option for overcoming this problem is to plant resistant varieties and that require extensive research about the biology and epidemiology of this virus, host, and insect interaction. The modern gene editing techniques can provide us good solutions and it can save the cotton industry in Pakistan.

Authors Contribution

Conceptualization: MH, SA, MSSC

Writing-review and editing: FM, SA, NH, MSSC, NK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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