



Destruction of cotton leaf curl virus and strategies to control it in future: A review

^a Usama Asif, ^b Hafiz Sabah-ud-din Mazhar, ^c Muhammad Zahir Ahsan*

^a Department of Plant Pathology, University of Agriculture (UAF), Faisalabad, Pakistan

^b Department of Plant Breeding and Genetics, University of Agriculture (UAF), Faisalabad, Pakistan

^c Cotton Research Station Sahiwal, Main Highway Sahiwal, Pakistan.

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*Corresponding Author's Email Address	ahsanzahir@gmail.com	Review Process: Double-blind peer review	
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ABSTRACT

Cotton is the backbone of Pakistan's economy and plays important role in it. Over the years cotton has faced many issues which effected the total yield of the crop and ultimately the economy of the country. The CLCuD is one of the devastating issues that cotton has faced in the past and suffering from it in the present. It is a viral disease and transmitted by white fly sp. *Bemisia tabaci* a sucking insect. The CLCuV belongs to the genus Begomovirus cause this disease and is difficult to control due to its recombination abilities i.e., ability to change the complete or partial genome, alternate host feature to travel in other crops due to mix cropping system and multiple strains in different regions of the world having a different genetic makeup. Scientists developed various techniques using modern and conventional approach to control the disease which showed significant impact on the controlling the CLCuV virus. Thus, considering vast benefits of these techniques, the study was undertaken to collect all the methods implemented by researchers to control the disease and increase the production of cotton fiber over the years.

Keywords: CLCuD, begomoviruses, CLCuV, gene editing.

INTRODUCTION: Cotton is the most important cash crop of Pakistan and is also considered as the backbone of Pakistan's economy (Ahmad *et al.*, 2010; Ali *et al.*, 2019). In gross domestic product of country, the share of cotton is 0.8%, as well as its agriculture value contribution is 4.1%. In Pakistan during 2019-20, the production of cotton is persisted moderate with 9.1 million bales. However as compared with 2018-19 there was a fall of 17.5% i.e., 11.96 million bales. The area of cultivation was increased by 6.5% but the overall output decreased in 2019-20 (Jatoi, 2021). Pakistan is the 5th largest producer of cotton after China, India, Brazil and USA (Abbas *et al.*, 2020). The global cotton production in 2018-19 is 118.5 million bales which is 4.2% lower than previous year (Zafar *et al.*, 2020). The genus *Gossypium* has 52 species but only *G. hirsutum*, *G. arboreum*, *G. barbadense*, *G. herbaceum* have the commercial importance (Saeed *et al.*, 2018). The tetraploid species *G. hirsutum* (often mentioned to as upland cotton) and *G. barbadense* (known as Pima/Egyptian cotton), have their Centre of origin in central America and northern Peru, respectively. *G. hirsutum* is the most widely cultivated species due to the quality of fibers its produces, which is long with a thick secondary wall, giving its strength. The diploid species *G. arboreum* and *G. herbaceum* are native to old world (OW) but the fiber they produced is not good as highly by processors. However, *G. arboreum* producing a low quality fiber resistant to CLCuV and is thus still an important profitable species (Sattar *et al.*, 2013). In addition, *Gossypium hirsutum* shares 90% in the total world production of cotton with respect to yield and quality. However, *G. barbadense* shares 8% this, ranked 2nd Whereas *G. herbaceum* and *G. arboreum* shares 2% of the world cotton production (Hasan *et al.*, 2019). Cotton jassid, cotton aphid, thrips, spotted bollworm, pink bollworm, American bollworm, cotton mealy bug, pink bollworm, fall Army worm and Whitefly are the major insect pests which cause major

loss to cotton production. The enormous use of pesticide leads to the insecticidal resistance, pest resurgence that increase the situation. These chemicals kills aimlessly beneficial and harmful insects, and also make worse the environment and human health (Zafar *et al.*, 2020). The sucking insects' pests suck sap from tender parts of plants and ultimately reduce the plant vigor. The unattended damage above threshold level may result in wilting and shedding of leaves (Sarwar *et al.*, 2020). Thrips, jassid, whitefly and dusky cotton bug may cause 40-50% loss to cotton crop. Among these pathogens affecting cotton (CLCuV) Cotton Leaf Curl Virus is considered the most dangerous in the production of cotton (Sarwar, 2020; Chi *et al.*, 2021). Cotton leaf curl is a viral disease transmitted by the whitefly (*Bemisia tabaci* Genn.). Gemini virus, belonging to the genus Begomovirus (family Geminiviridae) (Aslam Khan and Ali Khan, 2000). Gemini virus has 3 subgroups (Akhtar *et al.*, 2002). Cotton leaf curl disease first reported in Nigeria in 1912 later in Tanzania in 1926 and Sudan in 1934 respectively. In Pakistan, this disease was first reported from Tiba Sultan near district Multan in 1967. The disease did not receive much attention in beginning due to its casual occurrence and minor economic importance. Since 1987 it has become a serious threat to Pakistan cotton crop (Ahmad, 2001). This contagious disease hit area 97,580 hectares with a loss of 543,294 bales of cotton during 1992-93 season in the Punjab (Aslam, 2000). In Pakistan CLCuV appeared in epidemic form in 1990s named as emergence of latest strain 'Multan strain'. In 2001 a new strain of this virus was noticed in Burewala was named CLCuBuV (Cotton leaf curl virus Burewala) (Mahmood *et al.*, 2003). All resistant genotype of upland cotton became susceptible to this new viral strain (Saeed *et al.*, 2019). Accordingly, the strain of Burewala considers as a recombinant of Multan Strain's CLCuV as well as Kokhran strains. In Pakistan the disease averagely reduces plant height (40.6%), boll weight

(33.8%), and number of boll of plants (72.5%), fiber length (3.44%), fiber strength (10%), and elongation percentage up to (10%) (Abbas *et al.*, 2020). Generally, the disease lowers 30% biological yield of cotton crop in Pakistani fields (Naveed *et al.*, 2020).

Causal agent of CLCuD: The main origin of disease is virus, but it is transmitted in plants by an insect of the Phylum Arthropoda, Order Hemiptera and Family Aleyrodidae Whitefly (Carvalho *et al.*, 2017). Whitefly has various species out of which *Bemisia tabaci* is the vector for the CLCuV commonly known as Silverleaf whitefly (Fontenele *et al.*, 2017; Masood and Briddon, 2018). *B.tabaci* are considered the world's most dangerous species of whitefly and causes million dollars damage to cotton crop every year mainly because of its multiple strains present and its recombination ability to make new combinations of strains (Polston *et al.*, 2014; Jalal Shah *et al.*, 2020). It also has alternate host abilities and can cause a major problem to people using mix farming system as it can transfer to other crops like okra and tomato (Horowitz and Ishaaya, 2014; Rahman *et al.*, 2017). It is not only the source of transmission, but it also effects the growth of the plant by directly feeding phloem sap and indirectly by the excretion of honeydew on the plant which serve as a ideal condition for the growth of fungus on the plants (Ghanim, 2014; Zhu *et al.*, 2016; Qadir *et al.*, 2019). Gemini viruses are present in humid and high temperature zones mainly and are consist of nine genera. Gemini viruses have a geminate shape and consist of encapsulated single stranded circular genome (Xie *et al.*, 2010). Begomo viruses of the Family Gemini viridae induce the CLCuD in cotton (Malik *et al.*, 2014). Begomo viruses are consists of two types of genomes i.e. monopartite and bipartite. Monopartite genome consisting of a single nucleic acid molecule and have further two classes of satellite molecules i.e. alphasatellites and betasatellites (Xie *et al.*, 2010; Sohrab *et al.*, 2014). Alphasatellites are responsible for symptomatic induction and autonomous replication of the virus while betasatellites are symptom modulating satellites the need helper virus for the movement and multiplication (Zubair *et al.*, 2017). Monopartite genome of the begomo viruses is responsible for the encoding of 6 genes related to coat protein, replication enhancer and transcription activation, two in the sense orientation and two in the complimentary sense orientation (Wang *et al.*, 2016). Bipartite DNA genome consist of two single stranded and circular shape DNA i.e. DNA-A and DNA-B. six proteins are encoded by DNA-A while two are encoded by DNA-B respectively. There are different strains of begomo virus complexes effecting cotton crop virus of Multan (CLCuMuV), virus of Bangalore (CLCuBaV), Kokhran virus (CLCu-KoV), virus of Gezira (CLCuGeV), virus of Alabad (CLCuAlV), virus of Burewala (CLCuBuV) virus Shahdadpur (CLCuShV) (Amrao *et al.*, 2010; Iqbal *et al.*, 2012; Varsani *et al.*, 2017). Burewala strain of the virus is the new recombinant strain of the virus (Akbar *et al.*, 2012) and it effected all the previously cultivated resistant cotton varieties of *G.hirsutum* present in Pakistan by overcoming the TraP resistance and not expressing the same amino acid sequence (Saleem *et al.*, 2016). It is reported that Burewala strain has the similarity in genome with okra leaf curl virus genome (Hameed *et al.*, 2014). Sequence of the virus vary in region to region mean virus has a different strain in India, Punjab and Sindh province of Pakistan (Akhtar *et al.*, 2014).

Symptoms: Cotton leaf curl disease (CLCuV) infected plants may

show a wide range of symptoms depending upon the severity of disease. Cotton leaf curl virus disease characteristically on leaves. The primary symptoms of (CLCuV) is the Veins of leaves becomes thickened and more prominent on the lower side of the leaves. Thickening of small veins which is characterized by small beads like modifications on the leaves, is a common feature under our conditions. The irregular thickening slowly extends and coalesce to form a continuous reticulation of the small veins. Frequently there is emergence of the outgrowth, resembling small leaves on the lower sides of the leaves. These outgrowths are known as leaf "enations.". At the seedling stage, the appearance of the disease was so serious that retarded flowering, ball formation, maturation, and dramatically reduced the fiber quality and cotton seed yield (Nawaz *et al.*, 2019).

Disease rating scale: The resistant level is normally described on the severity basis of disease symptoms which may be at any plant stage, inoculation pressure and growth conditions. The rating scale used numerically in different crops (tomatoes, chilies, tobacco etc.) similarly in cotton with small modifications. The disease scales that had been used in cotton is based on severity index (SI) and percent disease incidence (PDI %). The formula used to calculate these two parameters also used yield data to correlate it with SI and PDI %. According to this formula individual symptomatic plant rating for every genotype are summed up and divided by number of infected plants to calculate the corresponding SI. This method of calculating disease incidence has been utilized in tea plant for blister blight, in chilies for fruit rot, mildew and citrus for citrus canker.

Epidemiology: Different climatic factor such as temperature, light, sex of white flies, wind, RH (%), rainfall and plant age affect the incidence and development of the CLCuV (Fahad *et al.*, 2017). if rainfall occur just before the seedling stage, then the population of the vector whitefly (*Bemisia tabaci*) will increase due to the increase in food sources. Primary site of the infection established when infected whitefly infect the cotton field (Iqbal *et al.*, 2014). Secondary infection occurs by spread of virus from the primary infection sources through additional vectors that enter in cotton field during whole growing season. Non-significant correlation was found at minimum air temperature, Relative humidity at 5 pm, velocity of wind, rainfall, sunshine, and whitefly population on thirteen mutant varieties and negative non-significant correlation was found between minimum air temperature and wind velocity at (8.am) for the development of the cotton leaf curl disease. There is a non-significant correlation of whitefly population with the disease development. Regression analysis used the on weekly air temperature (maximum and minimum), rainfall, relative humidity, and wind movement relationship with % plant infection by CLCuV on eight varieties of cotton. Disease infestation increased in the range of maximum and minimum temperature of 33-45°C and 25-30°C respectively. They also reported poor correlation of weekly rainfall and humidity with disease development and non-significant between CLCuD and whitefly population on all varieties studied. At the seedling stage, the appearance of the disease is so serious that retarded flowering, boll formation, maturation and dramatically reduced the fiber quality and cotton seed yield.

Molecular approach: By the development in Molecular techniques it is now easy to overcome and control CLCuV by using these techniques (Hassan *et al.*, 2015).

Transgenic varieties: Transgenic traits give growers beneficial

tools to manage weed insects, pests, and different abiotic stresses. Two important insects' pests group plant bugs (*Lygus spp.*) and thrips (*Thrips spp*) crash the cotton production and not controlled by current transgenic cotton varieties. There are several species of the thrips and bugs infesting the cotton. The study showed that MON 88702 (transgenic variety) proved to be resistant against bugs and thrips. MON 88702 is free from injury symptoms and provide significant reduction in number of lygus nymphs and yield production also increased. MON 88702 demonstrated less injury resulted in significant yield production and tolerance to herbicides as compared with its non-transgenic near isolines. Such a level of efficacy suggests that MON 88702, when incorporated into existing IPM programs could become an important tool for the management of lygus and thrips with existing chemical-based strategies in cotton Agro system (Akbar *et al.*, 2019).

The BC1 gene: Study reported that BC1 gene was used in the antisense rotation to develop a transgenic cotton variety. Bacterial mediated transformation method was used to insert the BC1 gene. First the gene was inserted in the bacteria (*Agrobacterium tumefaciens*) using 35S promoter of the Cauliflower mosaic virus and nopaline synthase as a terminator, using the molecular techniques the gene was inserted in the plants and confirmed by Polymerase chain reactions (PCR). The developed plants were resistant to the CLCuV disease and showed no signs of the disease even in the peak days of the attack to other crops. These results proved that use of transgenic traits to develop the transgenic varieties is the need of the hour and can be used to tackle the diseases (Sohrab *et al.*, 2016).

RNAi silencing of whitefly: The study reported that RNA-interference (RNAi) was used to knockdown the whitefly (*B.tabaci* species). it acted as immunity and knockdown the insects when the sequence was recognized, its efficacy was tested on bio assayed insects after a regular interval of time Six genes were selected from the gut of whitefly and results showed that RNAi silencing can be useful to weak insects and make it easy for insecticides to knockdown them (Vyas *et al.*, 2017).

The V2 RNAi construct: The study was conducted against the V2 gene of the begomo viruses which is responsible for the movement of the virus in plant cells. RNAi silencing technique used to tackle the virus in the transgenic varieties. Top performing cultivars were selected, and shoot apex cut method was used for the transformation of the gene which was cloned under 35S promoter. Different molecular techniques were used to confirm the transformation in the successive generations of plants like PCR, qPCR, FISH and karyotyping. The developed plants showed significant differences with the wild types in tackling the virus attacks when challenged with the whitefly attack and it was recommended to use these plants against virus (Yasmeen *et al.*, 2016).

Antisense RNA: Antisense technology is a tool that is used for the inhibition of the gene expression. The principal that behind it is that antisense nucleic acid sequence base pairs with its complementary sense RNA strand and prevent it from being translated into the protein (Amudha and Balasubramani, 2010). Antisense RNA technology is was used to develop transgenic plant resistance to CLCuD. It is most used and biotechnology and genetic engineering to incorporate resistance gene into the commercially profitable crop *G. arboreum* is resistance against the CLCuV and several other bacterial and fungal diseases. By

using genetic engineering *G. arboreum* has been used for the incorporation and isolation of the resistance gene into the susceptible cultivars. However genetic variation is insufficient in *G. arboreum* (Niu *et al.*, 2008). Pathogen derived resistance (PDR) is used to protect the plant against the several viruses lacking the natural disease resistance. By making use of transcriptional control two truncated form of replicase (*tACI*) gene expressing C-T terminal 783bp (3'ACI) and N- terminal 669bp (5'ACI) nt was introduced via cloning into *G. hirsutum*. This gene inhibits the replication of DNA- β satellites and viral genomes. To combat CLCuV, a strain LBA 4404 *agrobacterium tumefaciens* was used via interference technology in transgenic cotton (Briddon and Markham, 2000). When these transformed plants are compared with non – transformed control plants the over expression of nucleotide develop resistance by inhibiting β satellite and viral genomic viral genomic DNA components. Northern blot hybridization showed high transgene expression in late and early growth stages (Farooq *et al.*, 2014).

Pathogen derived resistance: Virus resistant can be developed by using pathogen derived resistance PDR. PDR based on cross protection and antisense approach has long been employed in the management of the viral diseases in plants. In PDR, a complete or viral gene is introduced into the host plant that later block the replication process of that virus. Conversely, because of the induction of the gene silencing by the viruses PDR is not found to be effective against these viruses (Yousaf *et al.*, 2013). It is divided into two categories with or without protein expression. Replication associated protein (Rep) is essential and play a key role to replicate the virus inside the host cell (Su *et al.*, 2015). It is also involved in the synthesis of viral component and in host cell replication by bringing the cell into S phase. This protein also used in for the induction of the resistance to control the viral infection. SiRNA strategy is used to interfere the structure of the protein (Khan and Jacob, 2015). If Rep protein structure is compressed, then viral component cannot be made. However not any commercial variety is arrived since by using this technology. Against CLCuV currently, intragenic region of *Ageratum conyzoides* are transformed but transgenic are still under trail (Nagata *et al.*, 2015).

Nonpathogenic derive resistance: Development of transgenic cotton varieties by using nonpathogenic derived resistance are in progress. To induce resistance against the various diseases by the introduction of the genes from the host or non-host plant. For example, introduction of genes responsible for DNA binding protein, coat binding protein, providing antiviral antibodies, etc., in plant to develop resistance against CLCuV. The first report of the development of transgenic resistance against plant virus involved the expression of *Tobacco mosaic virus (TMV)* derived viral coat protein (*CP*) gene and this strategy was later used against the Gemini virus (Khan and Jacob, 2015). A mechanism has been explored in mosses and ferns that give our resistance against the phytophagous insects. For example, a protein *Tma12* was identified in ferns that provide resistance against the whitefly. This gene encoding protein was developed in cotton CV. Coker-312. Only one cotton line has been shown to increase resistance (>99%) against whitefly (Shukla *et al.*, 2016). Hence this protein can be used in future to induce resistance against the whitefly and many other plant species.

Cell death induction: Modern molecular technique can be potentially used against the CLCuVD and its viral causal agents.

In transgenic plant, to inhibit the replication of Gemini viruses this approach has been used. It was developed by using *Bacillus amyloliquefaciens* derived combined action of barstar and barnase protein. Barstars acts suppresses barnase activity which is ribonuclease (RNAase). Barstar and barnase protein express at the same levels when there is no Gemini virus infection for limiting the RNAase infection. This approach successfully used against the Tomato leaf curl new Delhi virus *ToLCNDV* (Vanderschuren *et al.*, 2007). Under phloem promotor restriction of the whitefly population expressing the insecticidal gene in transgenic tobacco plant (Javaid *et al.*, 2016). This approach can be used in future to protect the cotton against gemnivirus.

Conventional approach: Non cultivated cotton species were continuously affected by begomo viruses and associated satellites, and they are like as reservoirs for the disease CLCuD. These results provide the concern of begomo viruses and associated satellites in New World cotton species introduces in the old civilization. This concludes that associated satellite species are maintained by non-cultivated cotton species and when given a suitable environment, it will spread (Shakir *et al.*, 2019).

Interspecific crossing: In the current scenario, there can be a chance of transferring genes to resist the disease named Cotton Leaf Curl Virus Disease (CLCuD) from *Gossypium Arboreum* having chromosome number ($2n = 26$) cv 15-Mollisoni into *G. hirsutum* ($2n = 52$) cv CRSM-38 with the help of conventional breeding. Then comes the process of cytology of the two progenies that we got from the direct and reciprocal cross of *G. arboretum* and *G. hirsutum*. This results in F1 progenies which completely put a resistance for the disease (CLCuD) (Iqbal *et al.*, 2003). In Reciprocal crossing, the disease incidence was more as compared in direct cross. In Cytological studies, disease resistant plants such as sterile and fertile plants, showed that number of univalent and multivalent were high in BC1. But it was low in BC2 with shy bearing plants. Due to bivalents, most of the plants showed normal bearing ability in BC3. Two techniques were carried out named as hybridization and Boll shedding. Hybridization technique was performed manually between an artificial autotetraploid of *G. arboreum* and allotetraploid *G. hirsutum* under field conditions. The technique Boll shedding was controlled by application of exogenous hormones, 50 mg/L, gibberellic acid and 100 mg/L naphthalene acetic acid. Percentage pollen viability in F1 hybrid was 1.90% in 2 (*G. arboreum*) *G. hirsutum* and 2.38% in *G. hirsutum*. In cytological studies of newly grown buds from F1 were sterile. In F1 hybrids, resistance against disease were determined through grafting by using the hybrid plant as scion. The stock plant containing a virus was tested under greenhouse and field environments (Aslam, 2000). Cotton hybrids of F1 showed resistance for the disease CLCuD. Which results in transferring genes from the allotetraploid *G. hirsutum* carried out with the help of conventional breeding. The study showed that conventional breeding can prove to be a long-term treatment for the tackling of the disease (Nazeer *et al.*, 2014).

Insecticide's effect: From the survey performed, the data which was collected showing the effects of insecticides at their suggested doses showed the control of insect's pests statistically while comparing with the control.

Population of insect's pests: When the crop field will remain

unsprayed results in increase in pest's density. While talking about comparative analysis of field, monocrotophos and endosulfan are best insecticides to control cotton bollworms (Shahid *et al.*, 2014).

Population of natural enemies: The insights showed that beneficial insects like coccinellid beetle *coccinella septumpunctata* (Linnaeus), hover fly *Eupeodes confrater* (Wiedemann), and bigeyed Bug *Geocoris ochropterus* (Fiber) were not affected and there were no differences in their population (Sarwar and Sattar, 2016). Two kind of biotypes *B. tabaci* were detected and named as B Biotypes and Q biotypes. From medium to high level of resistance to 2 neonicotinoids were establishes in both Q and B biotypes (28–1900 -fold to imidacloprid, 29-1200-fold to thiamethoxam). Moderate to high level of resistance to alpha-cypermethrin (22-610-fold)) were detected in both Q and B biotypes. Both Q and B biotypes of *B. tabaci* have high levels of resistance to imidacloprid and thiamethoxam. The most effective insecticides in the population against *B. tabaci* named "abamectin" (Wang *et al.*, 2010).

CONCLUSION

Eradication or control of CLCuV is the biggest hurdle for the scientists and researchers of the world. The virus is highly dangerous because of its abilities to overcome the resistance by making new viral strains and changing host in order to survive therefore it is very difficult to control it in mix cropping system which is normally practiced in the subcontinent i.e. India and Pakistan. After the outbreak of the virus first time in the world researchers deployed different conventional and modern methods to control it but after some time the virus overcome the resistance and destroyed the cotton crop every year. The virus has different strains in different parts of the world, and it is still evolving which makes it difficult for the researchers to control it. The production of the cotton is decreasing on yearly basis due to vast genetic makeup of CLCuV and on the other side current cultivars have narrow genetic makeup and it's not possible for the current cultivars to overcome it. In light of the literature, we come to know that the resistance used till now is either taken from different cultivars of *G. hirsutum* or a single layered resistance is used to stop the virus which proved less effective for the long period of time. Genome of *G. arboreum* is not exploited completely because of the other low qualities traits but its genome can prove beneficial in the future if exploited properly to look for the resistance genes. In future the molecular approaches like genome editing, CRISPR/Cas, QTLs, markers assisted breeding and many more must be used to implant multilayered resistance in cotton to survive the CLCuV outbreak. Conventional approaches can be used to resist the virus like extensive crossing of cotton varieties with the wild species to somehow bring out the resistance genes in the desired cultivar and by using different agronomical approaches and restricting the area of the virus. Conventional and modern methods should be combined to introduce a multilayered hybrid kind of a resistance in the crop and there is no other way stopping the virus. Farmers and researchers should be aware about the disease outcomes and stop the distribution of susceptible varieties in the different parts of the world and should follow strict quarantine measures on the borders in order to stop disease from spreading. Mix cropping system should be avoided so that virus cannot travel along different host ultimately stopping virus movement.

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