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CYTOGENETICS OF COTTON LEAF CURL VIRUS, ITS CAUSES, SYMPTOMS AND MANAGEMENT STRATEGIES IN COTTON

MUHAMMAD ASIM BHUTTA[1]. HAFEEZ UR REHMAN[1]. UMAR FAROOQ[1]. MUHAMMAD ZUBAIR ISHAQ[2]

[1]Cotton Research Station Sahiwal, Pakistan
[2]Department of Plant Breeding and Genetics, Faculty of Agricultural Science & Technology, Bahauddin Zakariya University Multan Pakistan
Corresponding author Email: bhuttabreeder@gmail.com.

Abstract

The production of Cotton "Gossypium hirsutum" is facing severe challenges in Pakistan, due to many biotic and a-biotic factors. However still it is considered as a significant cash crop in the country. While digging deep, the severe threat to cotton productivity in Pakistan is rooted in the "Cotton leaf curl virus (CLCuV)." Such a virus belongs to the genus Begomovirus of the Geminiviridae family and whitefly Bemisia tabaci is responsible for its transmission. CLCu, specific indications are veins swelling, curling as well as enations formation under the leaf of an infected plant. By the emergence of a recombinant strain derived from CLCuMuV and CLCuKoV named Cotton leaf curl Burewal virus (CLCuBuV), the disease spread in epidemic form in-country during 2006-07 while breaking all the resistance previously present in Gossypium hirsutum genotypes. The wild species such as "Gossypium anomalum, Gossypium capitisviridis, Gossypium sticksii, Gossypium somalense, Gossypium longicalyx, Gossypium gossypioides, Gossypium laxum and Gossypium areysianum" are a good source of resistance genes against CLCuV and can be used in interspecific hybridization for gene transfer through conventional breeding. Moreover, the use of advanced biotechnology tools such as marker-assisted selection and transgenic approaches could help develop resistance against this disease.

Keywords: Gossypium, CLCuD, B. tabaci, Begomoviruses

INTRODUCTION

In Gross Domestic Product of the country, the share of cotton is 0.8%, as well as its agriculture value addition contribution, is 4.5%. According to reports, during the fiscal year of 2019, the production of cotton persisted moderate with 9.861 million bales. However, as compared with the show in 2018, there was a fall of 17.5%, with11.96 million bales (Anonymous, 2019). This decline in production of cotton was due to rapidly changing climate in which number of biotic, a biotic factors and pathogens are causing production losses in cotton. Among these pathogens affecting cotton *(CLCuV)* is considered the most hazardous in the production of cotton. This virus was rooted in Nigeria in 1912 on wild species of cotton *Gossypium Vitifolia* and *Gossypium Peruvianum.* The primary cause of this disease is a virus whose transmission source is whitefly (*Bemisia tabaci*) (Nogia *et al.,* 2014). Once acquired by the Whitefly (*B. tabaci)*, CLCuV is retained throughout its life cycle, and it starts replicating in the nucleus of the infected plant cell. In Pakistan, CLCuV first reported in Multan in 1967 (Hussain, 1975). In Epidemic form, CLCuV appeared in Pakistan as the emergence of a new strain named 'Multan strain' in the 1990s. In 2001 by the emergence of Burewala strain in Vehari, the resistance was once again break in varieties resistant to the strain of Multan (Amrao *et al.,* 2010). Accordingly, the strain of Burewala considers a recombinant of Multan strain's CLCuV as well as Khokhran strain's CLCuV (Hina *et al.,* 2012). Yield losses in Pakistan due to CLCuV measures up to 30% every year (Ashraf *et al.,* 2013). The Punjab Province of Pakistan faced 543,294 bales yield

losses during 92-93 (Aslam and Gillani, 2000).

Classification of Geminiviruses: The plant pathogens Geminiviruses belong to family *Geminiviridae* and having a genome comprising of single-stranded DNA (Gutierrez, 2006). Geminiviruses are habitant of humid to warm temperature zones as well as weed's broad range, and additionally, wild plants are their host. Geminiviruses are comprised of nine genera *Begomovirus, Curtovirus, Mastrevirus, Topocuvirus, Becurtovirus, Turncurtovirus, Eragrovirus, Grablovirus, and Capulavirus* (Versani *et al.,* 1831).

Genomic Architecture of Begomiviruses:

Its vector whitefly, Bemisia tabaci, mainly transmits Begomovirus. Whiteflies emerged universally throughout the globe in any place they find their *B. tabaci* vector populations as well as considers the high risk to cotton crops (Varma *et al.,* 2003). Begomoviruses have an either bipartite or monopartite genome. Bipartite are two circular, single-stranded DNA known as DNA-A and DNA-B Figure.1 (Lazrowitz, 1987). At the same time, monopartite tend to be DNA-A of bipartite *Begomovirus* genomes' equivalent. The DNA-A component encodes six proteins, and the DNA-B segment codes two proteins. Every element ranges from 2.5 - 2.8 kbp in size. Autonomous replication is present in DNA-A component and produces proteins liable for compound encapsidation (Coat Protein), replication enhancer protein (REn), viral replication, management of gene expression as well as suppression of host defense mediated through gene silencing (transcriptional activator (TrAP) protein (Iqbal *et al.,* 2011). The development of symptoms had been linked to the protein encoded with C4 begomoviruses protein (Lazarowitz *et al.,* 1992). The nucleotide sequence of both DNAs is different except for the common region (C.R.), which is composed of 200 nucleotide base pairs (Hanley-Bowdoin *et al.,* 1999). C.R. includes a conserved string, 5'-TAA TATTAC-3' proven as a part of all Geminiviruses (Lazarowitz, 1987). Virus assembly is controlled by coat protein (C.P.) (Padidam *et al.,* 1996). Genes BV1 and BC1 are present on DNA-B, which take part in viral movement (Lazarowitz, 1992; Stanely and Townsend, 1985). Bipartite Begomoviruses additionally includes betasatellites; those are accountable for the seriousness of the infection. Similarly, it needs DNA A towards replica as well as encapsidation (Mansoor *et al.,* 2003). The regions of satellites take associate virus for a replica (Mayo *et al.,* 2005). However, the alpha satellite may be relating to the CLCuD complex (Briddon and Stanley, 2006). Duplication of Begomoviruses commences through transforming the only stranded DNA into double-stranded DNA. Then by using this double-stranded DNA as a template, it produces single-stranded DNA using a procedure identified as rolling circle replica (Amudha *et al.,* 2011). A new strain of the Cotton leaf curl virus was originated by the recombination of cotton leaf curl Multan Virus (CLCuMuV) and cotton leaf curl Kokhran Virus (CLCuKoV) that snap resistance as part of established cotton types (Amrao *et al.,* 2010). CLCuMuV, as well as CLCuKoV Recombinant, is revealed in Figure 2. The difference between the newly emerged Burewala strain and Multan strain resides as part of the beta satellite region difference of two varieties, i.e., CLCuMBBUR maintained ~80nt in SCR region taken from tomato beta satellite (Amin *et al.,* 2006).

Symptoms and disease rating scale:

Symptoms of cotton leaf curl virus (CLCuD) on an infected plant depends on the degree of the infection (Farooq *et al.,* 2011). Typical signs of (CLCuD) consist of thickening and swelling of veins, leaf curling as well as the production of enations in leaf's bottom side as shown in Figure 3 (Harrison *et al.,* 1997; Mansoor *et al.,* 1997). The infected plant produces a couple of categories of thickening, minor and significant vein thickening, which begins straight from the margins of the leaf furthermore expand inwards to make prominent thickened veins' network (Watkins, 1981). The infected plant shows stunted growth due to the reduction in intermodal distance and causes significant yield reduction in case of severe infection (Tanveer and Mirza, 1996). In the second meeting of CLCuD in 1996 (Anonymous, 1996) proposed a disease rating degree to discover the immunity or vulnerability of cotton traces to CLCuV. The proposed disorder rating scale with a few modifications by (Akhtar *et al.,* 2001; Akhtar, 2002) is shown in Table 4.

Strategies and tools to combat CLCuD

Acquisition of resistant genes from wild species through hybridization: The cultivated diploid species of cotton Gossypium arboreum and Gossypium herbaceum both tend to be resistant to CLCuD. Moreover, there are Gossypium diploid of 8 species types discovered as to CLCuD (Anonymous 2011). Such classes are (B1) Gossypium anomalum, (B3) Gossypium

capitisviridis, (E1) Gossypium sticksii, (E2) Gossypium somalense, (F1) Gossypium longicalyx, (D6) Gossypium gossypioides, (D9) Gossypium laxum as well as (E3) Gossypium areysianum. Due to embryo abortion after fertilization, gene transfer from wild species by conventional means has rarely been unsuccessful. Interspecific crossing regarding diploid classes along with upland cotton ends up with sterile F1 hybrids. Accordingly, for the production of hexaploids, such sterile hybrids must be addressed with colchicine (Joshi and Johri, 1972). For the successful boll setting of this interspecific cross, we have to apply Gibberalic acid to overcome shedding (Liang and Son, 1982 and Liang *et al.*, 1978). A BC2 population showed resistance to CLCuD. It was originated by manually hybridizing an allotetraploid G. hirsutum with an autotetraploid of G. arboreum L under field conditions (Ahmad *et al.*, 2011). These findings show the feasibility of achieving CLCuD resistance through conventional breeding by resistance gene transfer from diploid species by interspecific hybridization.

Biotechnology approaches against CLCuD using molecular marker techniques

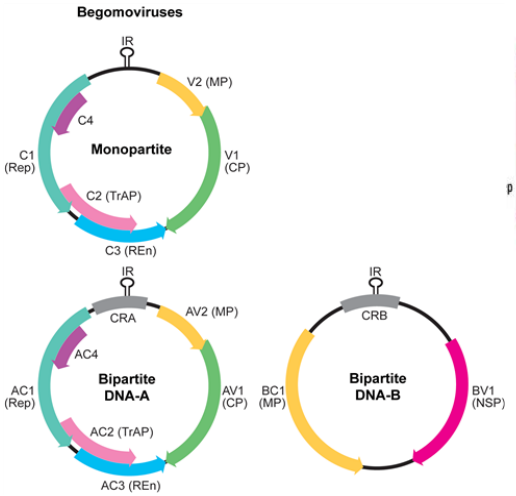
Because of certain limitations in conventional breeding and using the developments as part of biotechnological techniques, it is now effortless to overcome the cotton leaf curl virus through such sophisticated strategies (Farooq *et al.*, 2011). Attempts to classify DNA markers were made in Pakistan. However, the major handicap in identifying robust DNA markers was a small genetic diversity among the genetic material. Studies have been conducted using intra and interspecific crosses to map CLCuV and its viral causative agents' resistance to QTLs. RFLP markers have been used to identify DNA markers linked to disease resistance. Using RFLP a population of F2 was assessed and three DNA marker loci were found linked to resistant loci (Aslam *et al.*, 1999). Bulked segregant analysis (BSA) has been deployed pooling equal quantities of resistant and susceptible F2 plant genomic DNA into two different pools. A total of 520 random primers were examined on these bulks. Unfortunately, there was no identification of polymorphic RAPD priming. Then those RAPD primers were examined on experimental population parental genotypes. A total of 13 per cent were polymorphic amplicons. In trans phase, a RAPD marker was identified. Though OPO19460, OPQ14325, and OPY21080 (in coupling with recombination frequency of 05 per cent) were found to be associated with disease resistance (Rahman *et al.*, 2002). In another research CLCuD and its viral causative agents were screened for a total of 18 cotton genotypes. Just two genotypes CIM240 and CIM442 demonstrated resistance to the disease and its causative viruses (Mumtaz *et al.*, 2010). The intraspecific F2 population in one of the earlier studies. Crossing LRA5166 (resistant) and S12 (susceptible) was formed with hirsutum. This F2 population was screened using primers from RAPD, SSRs, and AFLP. Two parents were tested on a total of 225 RAPD primers. In total 11 parents were found to be polymorphic. To determine their association with disease resistance, these polymorphic primers were surveyed on F2 population. Of these, the resistance was linked to three marker loci "OPO19, OPQ14, and OPY2" (Rahman *et al.*, 2002). Studies reveals that the use of molecular markers related to cotton leaf curl virus disease resistance could be a helpful tool towards development of CLCuV resistant varieties (Farooq *et al.*, 2011).

Field management strategies

At the 1st stage of the CLCuV affected plant can be recouped by balanced use of fertilizers and yield losses could be minimized by maintaining plant population (Pervez et al., 2007). Disease resistance can be improved by decreasing the nitrogen application and improving use of potassium (K) as it strengthens the defense mechanism of the plant (Chang and Liang, 1978). Researchers revealed that late sown cotton was more vulnerable to CLCuV as compared to early sown crop (Iqbal et al., 2008). According to the suggestions by above mentioned results by researchers, cotton yield losses can be minimized by maintain proper plant population and plant can be recouped by adequate use of fertilizers especially potassium (K) based

Table 1. The disease rating scale for CLCuD

Symptoms	Disease Rating	Disease Index (%)	Disease Reaction
Absence of Symptoms	0	0	Immune
Thickening of a few small veins or the presence of leaf enations on 10 or fewer leaves of plants	1	0.1-1	Highly resistant
Thickening of a small group of veins	2	1.1-5	Resistant
Thickening of all veins but no leaf curling	3	5.1-10	Moderately resistant
Severe vein thickening and leaf curling on the top third of the plant	4	10.1-15	Moderately susceptible



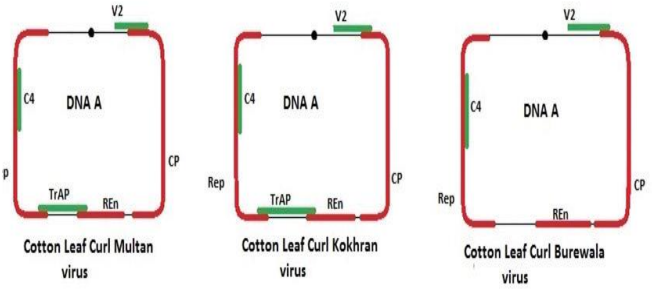


Fig. 2. (CLCUBuV)Recombinant of (CLCuMuV) and (CLCuKoV) (Amin et al., 2006).

Fig. 1. Genome organization of Begomovirus (Lazrowitz, 1987

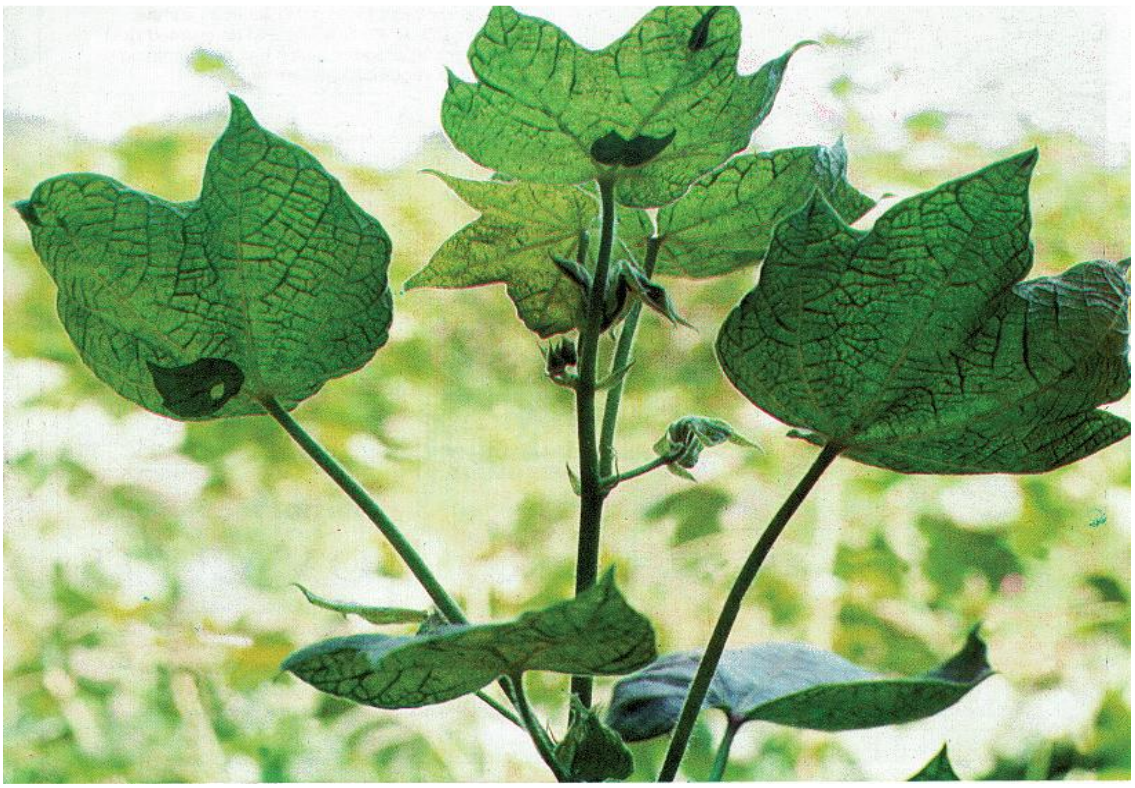


Fig. 3. Symptoms of CLCuV infected plant showing leaf curl, vein thickening and enations (Iqbal et al., 2014)

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