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Cotton Leaf Curl Virus (CLCuV) Disease in Pakistan: A Critical Review

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Abstract

Pakistan's cotton yield is in substantial threat due to cotton leaf curl disease. Overall yield is reduced as a consequence. In Pakistan, about 30% reduction in yield is caused per year. CLCuV belongs to the family Gemini virus and genus Begomovirus. CLCuV is a *Bemisia tabaci*, (whitefly) transmitted virus and is allied to the viral family Geminiviridae. In Pakistan there are about three strains of leaf curl virus Khokhran strain, Multan strain and Burewala strain. Many resistant varieties against khokhran strain and Multan stain has developed by scientists but no resistant variety against Burewala strain is developed. Burewala strain contains the sequences both from the Khokhran strain and Multan strain. There is not a single variety of *Gossypium hirsutum* which is resistant against Burewala strain of CLCuV. The effects of CLCuV on cotton plant comprise of enation, a situation by which upward cup shaped curling of cotton leaves and swelling of veins occurs. This cause damaging of lower part of the leaf which causes reduction in yield. The proliferation of tissues having chloroplast leads to more greening phenomena in diseased plants. Cotton belonging to the genus *Gossypium* comprises of more than 50 wild and domesticated species. Out of 50 species diploids are 45 while 5 are allotetraploid. The cultivated specie *Gossypium arboreum* is a great source of resistance against this specific cotton virus as there is not a single plant of *Gossypium arboreum* has shown the symptoms of this disease. The resistance can be developed through conventional breeding as the successful crosses of *Gossypium hirsutum* with *Gossypium arboreum*. As the rapid advancement in the field of biotechnology *Gossypium arboreum* exploitation as a resistant source and relocation of highly tolerance/resistance genes against CLCuV into highly susceptible genotypes with genetic engineering and other biotechnological techniques**.** Molecular markers can also be used against this disease by molecular markers gene mapping can be done easily and the resistant genes against CLCuV present in the *Gossypium arboreum* and other species of cotton can be isolated and transfer in the susceptible varieties.

1 Introduction

Pakistan's most outstanding cash and fiber crop is Cotton and it stand's as the second most important oil seed crop of the world (Farooq et al .,2014).Cotton (*Gossypium hirsutum*) belongs to the genus Gossypium and family Malvaceae (Azhar et al .,2013;Ali *et al.*,2010).Pakistan is on the 4th number in the production of cotton throughout the world and on the first number in exporting yarn (Aleem Ashraf *et al.*,2013).It contributes about 8.6 percent in agriculture and 1.8 percent in GDP of Pakistan (Ahmad *et al.*,2010).Cotton yield is affected by many factors such as disease ,insect attack and environmental factors but the most devastating agent which cause reduction in the yield of cotton in Pakistan is Cotton leaf curl virus(CLCuV) Nigeria, in 1912, was found to be attacked by this disease (Farquarson, 1912). Multan, in 1967, was the first reported area in Pakistan to be infested by this viral disease (Hussain, 1975). About 30 percent loss in the yield of cotton is caused by CLCuV in Pakistan every year (Ashraf *et al.*, 2013). 97,580 hectares of cotton area was affected leading to loss factor of 543,294 bales during 92-93 in the province of Punjab, Pakistan (Aslam and Gillani). CLCuV belongs to the family Gemini virus and genus Begomovirus (Akhtar *et al.*, 2005). CLCuV is a whitefly transmitted virus and is allied with viral family Geminiviridae. Gemini viruses are a diverse group of viruses which uses insect vector for their transmission. Gemini viruses have single stranded DNA (ssDNA) genome (Moffat, 1999). Geminiviruses are found mainly in warm and tropical climatic areas of Pakistan (Hameed *et al.*, 1994; Amudha et al). Currently gemini viruses On organization and composition base are alienated in 4 genera i.e. Begomovirus, Curtovirus, Topovirus and Mastrevirus. (Fauquet *et al.*, 2008; Stanley J, 2005). Studies have depicted responsibility of leaf curl disease on a Begomoviruses group called (CLCVs) are responsible for leaf curl disease in cotton crop and cause a huge loss on production. Important Strains of this group are CLCuV Multan virus, CLCuV Kokhran virus, CLCuV Burewala virus (Hina *et al.*, 2012). CLCuV is caused by monopartite begomoviruses that require a DNA satellite which should be disease-specific named CLCuM beta satellite (Briddon *et al.*, 2001; Mansoor *et al.*, 2003). In Pakistan Epidemic Attack of CLCuV started in early 1990s (known as "Multan strain") was managed by the development of resistant varieties through conventional breeding. However, in 2001 symptoms of the disease started to appear on resistant varieties of Multan strain as well due to emergence of new Strain (Burewala strain) (Amrao *et al.*, 2010). Burewala Strain of CLCu Disease is an enduring threat to cotton production of Pakistan. Cotton leaf curl burewala strain (ClCuBV) is a deviant that is formed of recombination among CLCuV Multan strain and CLCuV Khokhran strain (Hina *et al.*, 2012). At present no resistant tetrapolid genotype against Burewala strain has been observed (Iqbal *et al.*, 2014). In the year 1997 the disease incidence level was 32.4 percent and after it was gradually decreases due to the development of resistant verities against this disease, in the year 1998 the disease incidence level was 16.5 percent and it continue to decrease and was gradually decreases as in year 1999 was 14.3 percent and in 2001 it was minimum about 1.8 percent. In the year 2004 the disease incidence level becomes 40.7 due to the emergence of new strain the Burewala strain of CLCuV and in the year 2009 it achieves its maximum level of incidence 62.7 percent due to its vector and favorable environment for the disease .A minute proportional volume of disease is controlled by controlling its vector , adopting many improved growing techniques and use of balanced fertilizers so the disease incidence level comes from 62.7 to 35.8 percent in the year 2013(Department of .Pest warning and Quality Control of Pestiside, Punjab)

2 Symptoms and effects of CLCuV on Cotton Plant

Cotton leave's downward curling, their cup like structure formation called enations and swollen veins of leaves are few of the symptoms regarding CLCuD (Figure 1-4). Two types of thickenings of veins are observed on cotton plant; i) Major vein's thickening ii) Minor vein's thickening. Initaly the thickening is at leaf margins but with the increase in disease intensity it extends inward and form thickened veins network (Watkins, 1981). Young leaf's fine veins thickens and turn pale this depicts presence of minor type of vein thickening (Nour and Nour, 1964), Chloroplast deposited tissues get proliferated thus CLCuV effected genotypes appear darker than normal one's. (Sattar *et al.*, 2013). CLCuV has devastating Effects on the growth of cotton plant. It causes Reduction in Plant height (40.6%), Boll weight (33.8%) ginning outturn 3.9% and number of bolls(72.5%) per plant of Cotton (Mahmood et al .,1996). A savior decrease in staple

length, up to 3.44%, staple strength up to 10% and staple elongation %age occurs due to CLCuV (Ahmed .,1999).It has devastating effects on yield as yield is reduced at great extent. In 1991_1992 cotton production of Pakistan were maximum 12.4 million bales (MB) but in 1994 due to CLCuV cotton production reduced to 7.9 million bales from 12.4 million bales (Mahmood *et al.2013)***.** After it highly tolerant/resistant varieties against this disease got developed. CLCuV disease loses were minimized while cotton yield was also maintained between 8-11.5 MB. Tireless efforts of scientists eventually brought about highest crop production during the cropping session of 2004-05 i.e. 14.5 MB just before CLCuV struck again with a new Burewala Strain (Amin *et al.*, 2006). Devastating effects on crop were revealed by Burewala strain. Present studies showed that there is not any tetrapolid genotype of cotton completely resistant (Iqbal *et al.*, 2014).

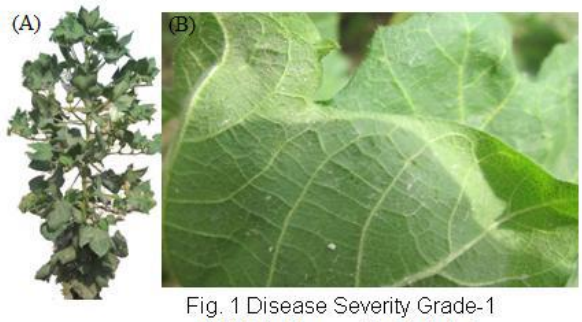

Fig. 1 Disease Severity Grade-1
A) Whole Plant B) Single leaf

3 Scale for CLCuV Intensity rating

$$\text{Percent disease tolerance} = \frac{\text{Total plants - diseased plants}}{\text{Total plants}} \times 100$$

The (PDT), percent disease tolerance calculation is done after the selection of a minimal 100 No. of plants in diagonal and counting of diseased plants determine the PDT.

Fig. 2 Disease Severity Grade 2
(A) Whole plant (B) Single Leaf

Severity Scale	Symptoms Visible	Conclusion
0	Symptoms are not present	Resistant
1	Thickening at initial level	Highly Tolerant
2	Thickening of the group of veins or top area affected	Tolerant
3	Thickening coupled with enation and 50% plant affected	Susceptible
4	Severe disease symptoms	Highly Susceptible

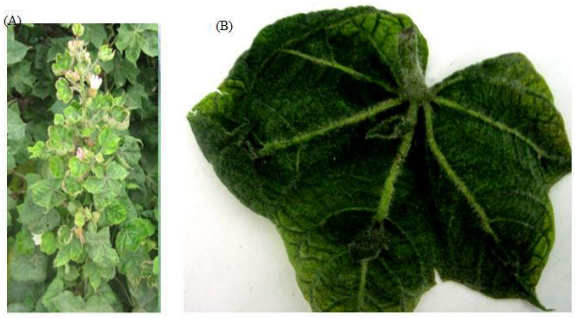

Fig. 3 Disease Severity Grade 3
(A) Whole Plant (B) Single Leaf

4 Photographic Profile of different severity Grades

The photographic demonstration clearly differentiates the different severity grade due to this viral disease.

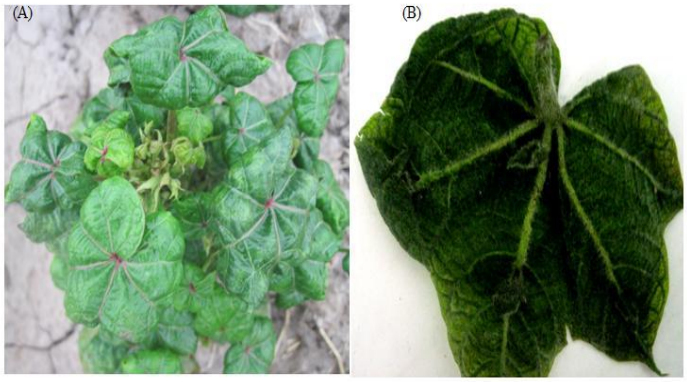

Fig. 4 Disease severity Grade 4
(A) Whole Plant (B) Single Leaf

5 Classification and way of transmission of Geminiviruses

Geminivirus are 2700–3000 nt weighing 1 or more components of single stranded circular DNA containing organisms which are usually transported through insect pest. (Moffat, 1999). Gemini viruses damage the crops and they are mainly spread due to the weak quarantine measures, lack of control of insect vectors and poor sanitation (Gray & Benerjee, 1999). On Structural, Host and insectal range Gemini viruses are alienated in 4 genera i.e. Begomovirus, Topocuvirus, Curtovirus & Mastrevirus (Rybicki *et al.*, 2000, Fauquet & Stanley, 2003, Stanley *et al.*, 2005).Monocotyledons infecting Mastrevirus's genome is monopartite having leaf hopper as a virus transmitting vector (Palmer and Rybicki, 1998). Curtovirus's monopartite genome use leaf- hopper as its vector while infecting dicotyledons only (Mansoor *et al.*, 2003). Similarly monopartitte genomic Topocovirus infect dicotyledons only but are of 2800 bp with similar vector as curtovirus (leaf hopper) (Briddon *et al.*, 1996).Begomovirus have both monopartite and bipartite genome. CLCuV is undoubtedly monopartite begomovirus having white fly as vector. A variety of crops are infected by these four genera of gemini viruses (Morales & Anderson, 2001 ; Mansoor *et al.*, 2003 b). Genus Begomovirus undoubtedly is most variable, most economically devastating and extremely geographically distributed genus among viruses (Muhammad Aslam* and Atif Ali Gilani., 2000).

6 Genome organization basis of Begomovirus Classification

On genpmic organization basis, Begomoviruse are alienated among 2 groups i.e. monpartite and bipartite. Begomovirus composed of genome, weighing near about 2600 nt each of 2 DNA molecules i.e. DNA-A and DNA-B are called bipartite whereas begomovirus having only single DNA molecule of 2800 nt i.e. DNA-A are called monopartite (Fauquet *et al.*, 2008). Monopartites have additional circular DNA of weight near about half then genomic one called alpha or beta –satellite. Beta-satellites cause the pathogenicity while no clear function is known for alpha-satellites regarding symptoms (Mansoor *et al.*, 1999 & Saunders *et al.*, 2008).). The beta-satellite has three major features a single βC1 gene, adenine rich sequence region and satellite conserved region containing stem-loop structure, which helps for the replication in gemini viruses (Hanley bowdoine *et al.*, 1999). In the Begomoviruse component DNA A is responsible for indigenous DNA multiplication, transmission through insects genetic expression control while DNA B component stands for encoding 2 genes for viral movement in plants (Rybicki *et al.*, 2000).

7 Cotton species a source of resistance Against CLCuV

Cotton belonging to *Gossypium* genus comprises of about 50 wild and domesticated species. 45 out of 50 are diploid while 5 are allotetraploid (Fryxell, 1979). Wild species are alienated in 8 genomes i.e. A - G and K whereas 4 cultivated species i.e. *Gossypium hirsutum* (cultivated species, tetraploid), *G. barbadense* (cultivated species, tetraploid), *G. arboreum* (cultivated, diploid) and *G. herbaceum* (cultivated, diploid).*G. hirsutum* and *G. barbadense* contributes more than 90 percent in the cotton production of the world. The two tetraploid cultivated species of cotton are susceptible to CLCuV while the two diploid species of cultivated cotton (*G. arboreum* and *G. herbaceum*) of A genome, were found to be free of virus. *G. hirsutum* being less in genetic diversity is not resistant towards the attack of this virus. Wild diploid species are resistant and this characteristic can be utilized towards the creation of resistant germplasm (Nazeer et al., 2014). Breeders are always in attempt to make the resistant varieties. The cultivated species having AD genome were found to be susceptible to CLCuV due to the presence of both begomovirus and betasatellite (Azhar *et al.*, 2013). Cotton's wild species are great resistance source against biotic (Living) and abiotic (Non living stresses) (Yik and

Birchfield, 1984) Some wild species of cotton G. stocksi, *G. harkensii*, *G. aridum* & *G. darwini*, are excellent drought tolerance sources while G. thurberii is frost tolerance source (Rooney *et al.*, 1991). The PCR and hybridization shows that viral molecules of CLCuD are absent in *G. arboreum* and *G. herbaceum*. The PCR results showed the presence of begomovirus and beta-satellite molecules in species belonging to D genome and in species belong to AD genome. In addition to PCR and Southern hybridization, technique efforts were also made by RCA (restriction analysis) technique for the detection of circular molecules of begomoviruses. The restriction analysis showed the absence of begomovirus and beta-satellites in *G. arboreum* and *G. herbaceum* but viral molecules of CLCuV were found to be present in the species belonging to D genome (Azhar *et al.*, 2013). Due to their characters, these wild species (diploids and tetraploids) are utilized in various hybridization programs (Mehetre *et al.*, 2003, Mehetre *et al.*, 2004). Surveys and study on large areas and number of plants of *G. arboreum* did not identify even a single plant showing symptoms of Leaf curl disease it means arboreum have some resistant genes against CLCuD (Briddon and Markham, 2000).

8 Genetic diversity and biotechnology a weapon against CLCuV

Maintaining genetic diversity is a great source of protection against a lot of diseases and pests (Van Esbroeck *et al.*, 1999). By the use of various indigenous and exotic germplasm a lot of varieties with enhanced productivity have been breed since Pakistan's independence. At present scientists are in search of any resistant/highly tolerant material as all existing one is greatly susceptible to Burewala strain. Some varieties are least susceptible against CLCuV so they can be a scored against CLCuV. It also provides an valuable supply of allelic variation that can be used to develop new favorable gene combinations (Rana1 *et al.*, 2005).By development in biotechnological techniques its easy now to overcome CLCuV by viruses cloning. (Farooq *et al.*, 2011). Not only a single plant of *Gossypium arboreum* shown the symptoms of CLCuV so *it* is considered CLCuD free and also from numerous other fungal and viral diseases (Briddon and Markham, 2000). Exploration of *Gossypium arboreum* has exploited for resistant gene isolation and its transfer into susceptible genotypes against CLCuV with the helf from genetic engineering and other biotechnological techniques (Farooq *et al.*, 2011).Molecular markers can also be used against this disease by molecular markers gene mapping can be done easily and the resistant genes against CLCuV present in the *Gossypium arboreum* and other species of cotton can be isolated and transfer in the susceptible varieties (Farooq *et al.*,2011). 3 DNA markers are found, that were associated and had alliance with CLCuV, evaluated by F2 genration through selective genotyping by RFLP (Kasschau and Carrington, 1998; Mikhail *et al.*, 2003).

9 Conclusion

Both diploid species of Cotton *G. arboretum* and *G. herbaceum* are resistant against Cotton leaf curl virus can be used as a source of resistant genes, and manipulation and introduction of these genes in cultivated tetraploid susceptible species. The vector of this disease *Bemisia tabaci* should also be controlled so that the minimum disease transfer occurs and more tolerant varieties against CLCuV should be grown. Genetic engineering, biotechnology and conventional methods of breeding should be used to overcome this threat to cotton production.

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