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AN OVERVIEW OF COTTON LEAF CURL VIRUS DISEASE, PERSISTANT CHALLENG FOR COTTON PRODUCTION

M. IQBAL, M. NAEEM, U. AZIZ, J. AFZAL and M. ALAM KHAN
The Islamia University of Bahawalpur, University College of Agriculture and Environmental Sciences,Pakistan

Abstract

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Cotton leaf curl virus (CLCuV) disease is crucial threat for cotton production in Pakistan. CLCuV belongs to genus *Begomovirus* of Geminiviridae family and is transmitted by *Bemisia tabaci*. Drastic reduction in yield was observed during 1994-95 and 2007-08 due to this disease. However in 1996-1997 CLCuD resistant varieties such as CIM 1100, CIM 448, CIM 446, MNH 554 and VH 53 were evolved but in 2006-07 with the development of recombinant specie (Cotton Leaf Curl Burewala virus) having sequence derived from CLCuMuV and CLCuKoV, disease spread in epidemic form breaking all of the available resistance in *Gossypium hirsutum* L. germplasm. The resistant sources against the new species of CLCuV are *Gossypium herbaceum* and *Gossypium arboreum* and eight wild diploid species which can be used to transfer resistant gene in upland cotton. Owing to unavailability of resistant varieties losses due to this disease can be minimized by adopting certain management strategies like early sowing, balanced use of fertilizer and eradication of host plants from field. Utilization of advanced bio-techniques such as marker assisted selection, RNAi and DNAi based genetic resistance may prove to be fruitful for the control of the disease.

In this review article we have tried to cover all aspects of cotton leaf curl disease and losses caused by this disease. Cotton leaf curl virus causes severe reduction in cotton production. Development of cotton leaf curl virus resistant varieties can diminish the damages of this disease. Balanced use of fertilizers and eradication of host plants and weeds can reduce the disease incidence. Previously scientists of CCRI Multan and different research organization have tried to incorporate resistant gene from *G. arborium* and *G. herbacium* by inter-specific crossing with *Gossypium hirsutum* L. Hexaploid population of above mentioned inter-specific cross was developed together with back crossing with upland cotton but success in this aspect is not significant. Secondly, amphidiploids of resistant diploid species followed by crossing as well as back crossing with upland cotton were studied but resistance obtained was not strong enough to combat this disease. We consider in future, that synthetic tetraploids should be developed by using resistant diploid species having "A" and D genome such as: 1. *Gossypium arboreum* × *Gossypium gossypioides;*2. *Gossypium hebraceum* × *Gossypium gossypioides;*3. *Gossypium arboreum* × *Gossypium laxum;*4. *Gossypium herbaceum* × *Gossypium laxum*

Key words: CLCuD, Resistance, Upland Cotton, Biotic stress, Whitefly, Gemini viruses

Introduction

Cotton (*Gossypium hirsutum* L.) is the most important cash crop of Pakistan, belongs to the Genus *Gossypium* of the family Malvaceae (Brubaker et al., 1999a). It accounts for 8.6 percent of the value added in agriculture and about 1.8 percent to GDP (Anonymous, 2011c). Many pathogens attack cotton plant and induce different diseases that cause severe losses in cotton production (Ahmad et al., 2011). Cotton Leaf Curl Virus Disease (CLCuD) is the most destructive disease causing huge losses to cotton production (Khan and Ahmad, 2005). First time cotton leaf curl disease was reported in Nigeria during 1912 (Farquarson, 1912). Later on reported from Tanzania and Sudan in 1926 and 1934 respectively (Bailey, 1934). In Pakistan cotton leaf curl virus was reported for the first time in 1967 near Multan (Hussain, 1975). CLCuVD is passeed on by its vector whitefly (*Bemisia tabaci*) and belongs to the genus *Begomovirus* family *Geminiviridae*, previously known as sub

Corresponding author: muhammadiqbal999@hotmail.com

group III (Hameed et al., 1994). According to the findings of Mahmood et al. (1996) CLCuD causes average reduction in plant height (40.6%), boll weight (33.8%) and number of bolls per plant (72.5%) in cotton cultivars. Ahmed (1999) showed that CLCuD can cause decrease in fiber length (3.44%), fiber strength (10%) and elongation percentage up to (10%).

Cotton Production of Pakistan was achieved ever maximum during 1991-1992 (12.4 million bales). Due to appearance of Cotton Leaf Curl Virus disease production was dropped to 7.9 million bales in 1994 (Mahmood et al., 2003). Later on tolerant varieties like CIM240 and MNH147 were released by Central Cotton Research Institute Multan (CCRI) and Cotton Research Station Multan (CRS) respectively. Losses due to CLCuD were minimized to restrained level and cotton yield remained in between 8-11.5 million bales. Consistent and distressing efforts of cotton breeders eventually lead to a bumper crop yield of 14.5 million bales in 2004-05 before the onset of a new and mutant species of CLCuD known as Burewala virus (Amin et al., 2006). Appearance of Burewala virus proved even worse to cotton industry raising losses to lowest level in 2007-08. Present review high lights the unavailability of completely resistant *Gossypium hirsutum* L. genotype against this race of CLCuD. It is eminent that Burewala race of CLCuD is an enduring threat to cotton production of Punjab Pakistan.

Genome Organization of Begomovirus

Family *Geminiviridae* comprised of genera i.e., *Mastreirus, Curtovirus Begomovirus and* (Seal et al., 2006; Farooq et al., 2011; Fauquet and Stanley, 2003) (Table 1). The genome of Mastrevirus is monopartite and virus is transmitted by its vector leafhopper and infects monocotyledonous plants (Palmer and Rybicki, 1998) (Table 1). The genome of Curtovirus is also monopartite and is transmitted by leafhopper but infects dicotyledonous plants (Mansoor et al., 2003b). Topocovirus also infects dicotyledonous plants having monopartitte genome (2800 bp) and is transmitted by leaf hopper (Briddon et al., 1996). Cotton leaf curl virus that is transmitted by whitefly belongs to *Begomovirus*, (Brown et al., 1995; Rybicki and Fauquet, 1998). South East Asia can be regarded as possible centre of origin of Geminiviruses owng to the existence of greatest molecular diversity in this region (Rehman and Faquest). The genomic components of the three genera of Geminiviridae are shown in Figure 1. DNA-A and DNA-B are two genomic components of bipartite *Begomoviruses,* (Monga et al., 2011). Both DNAs are different for the nucleotide sequences except for the region which is comprised of almost 200-nucleotides and is known as common region (CR) (does not exist) (Hanley-Bowdoin et al., 1999) (Table 1). CR is the part of intergenic region (IR) and contains a conserved sequence, 5'-TAATATTAC-3' which is found in all Geminiviruses (Lazarowitz, 1987 Table 1). The DNA-A harbors six genes that are responsible for DNA replication, control of gene expressions and insect transmission, whereas DNA-B contains two genes that are involved in movement of virus within the plants (Rybicki et al., 2000; Stanley, 1983 and Noueiry et al., 1994) (Table 1). DNA A and DNA B components possesses strictly distinct molecular histories evolved under different evolutionary pressures (Briddon et al 2010). AV1 or coat protein (CP) is required for virus assembly (Padidam et al., 1996) mutation of CP gene abolished infertility (Iqbal et al., 2012). (Rep) protein is involved in virus replication, C4 is involved in symptom development while its mutation caused reduced infertility (Iqbal et al., 2012) and TrAp is involved in transcriptional activation of viral genes (Lazarowitz, 1992). DNA-B contains two genes product, BV1 and BC1 that are involved in viral movement (Lazarowitz, 1992; Stanely and Townsend, 1985) (Table 1).

Bipartite Begomoviruses also involves betasatellites which enhances severity of disease, and requires CLCuD DNA A for replication and encapsidation (Mansoor et al., 2003b). Satellites are genetic components that are dependent on helper virus for replication (Mayo et al., 2005). About 200 betasatellites have been reported and have 45% sequence homology among each other. All betasatellites have three things in common i.e., a highly conserved region called satellite conserved region (SCR), an adenine rich region and single gene βC1, the product of βC1 is involved in inducing symptoms and may be involved in virus movement (Saunders et al., 2004; Cui et al., 2005; Saeed et al., 2005) (Table 1). In addition to original betasatellite of Cotton Leaf Curl Multan Virus its recombinant ($CLCuMB^{BUR}$) is prevailing at commercial cotton crop because of its ability to cross the host resistance barrier (Azhar et al 2012). Another important function of βC1 is suppression of RNA silencing effects also βC1 has a key role in viral pathogenesity (Tiwari et al., 2012). There in another satellite like molecule collectively known as alphasatellite and is found to be associated with CLCuD complex (Briddon and Stanley 2006). Mechanism of replication of Begomoviruses involves the conversion of ss-DNA into double stranded DNA (ds DNA) intermediates and then using dsDNA as a template to produce mature ssDNA genomes by a rolling circle replication mechanism (Amudha et al., 2011). Amin et al. (2006); Sharma and Rishi (2007) (Table 1), reported seven species of Begomoviruses so far, five of these species identified in Pakistan, one in India and one in Sudan. A new, recombinant species of Begomovirus, Cotton Leaf Curl Burrewala virus was evolved which was

Table 1
Brief description of genomic organization of various Genera of Geminiviridae family

Family	Genera	Vector	Infection	Genome Organization	DNA Composition	Gene Product	Function	Location on Genome
Eminiviridae (2700-3000 nt) Moffat 1999	Masterivirus	Leaf Hopper	Mono-cotyledenous Plants	Monopartite	~2700bp ss-DNA	MP, CP, Rep, RepA Proteins	(MP) Cell-Cell movement, (CP) Vector specificity Boulton et al., 1993	Virion strand, complementary sense strand, Boulton et al., 1993
		Palmer &Ribicky 1998						
	Curtovirus	Leaf Hopper	Dicotyledenous Plants	Monopartite	~2900bp	Capsid protein, MP, ss/ds DNA regulator, Rep Protein, Rep. enhancer, symp. Develp protein, pthogenicity associated protein	Cell-Cell movement, replication enhancing, symptom development,	3 proteins on Virion sense strand, 4 on complementary- sense strand
		Briddon et al., 1996					Stanley et al., 1992	
	Topocuvirus	Tree hopper	Dicot Plants	Monopartite	~2800bp			
		Briddon et al., 1996						
	Begomovirus	White Fly	Dicotyledenous Plants	Monopartite	DNA-A Like 2800 nt Fauquet et al., 2008 Beta Satellite or Alpha Stellite = 1400nt	Replication, Gene expression, White fly transmission, Systemic Infection (AV1, AV2, AC1, AC2, AC3, AC4)	Symptom development, cell-cell movement, replication enhancement	Virion sense strand, complementary-sense strand
					Mansoor 1999;	Saunder et al., 2008	Klute et al., 1996	
	Faquet& Stanly 2003 Rybicki et al., 2000 Stanly et al., 2005;Seal et al., 2006			Bipartite	DNA-A 2600nt	AV1, CP (Coat Protein) AV2	Virus assembly Cell to Cell Movement of Virus Padidam et al., 1996	Virion Sense Strand
		Brown et al., 1995; Rybicki and Fauquet, 1998						
					DNA-B 2600nt	AC1	Viral replication	
				Fauquet et al., 2008		AC3		
						AC2	Transcriptional activation of Viral genes	
						AC4	Symptom Development	
							Lazarowitz 1992	
						BV1	Viral Movement	Sense strand
						BC1		Complementary Sense strand
							Rybicki et al., 2000, Stanley 1983, Noueiry et al., 1994	Lazarowitz 1992, Stanly & Town send 1985

derived from cotton leaf curl Multan Virus (CLCuMuV) and Cotton Leaf Curl Kokhran Virus (CLCuKoV) and was found responsible for breaking resistance in existing cotton varieties (Amrao et al., 2010). Recombinant of (CLCuMuV) and (CLCuKoV) is shown in Figure 2. CLCuMuV and CLCuBuV differed from each other owing to the variation in the betasatellite region of two species i.e., CLCuMB^{BUR} carried ~80nt in SCR region derived from tomato betastellite (Amin et al., 2006). Transcription analysis of CLCuBuV and CLCuMB-^{BUR} didn't reveal any detectable variation when compared to earlier reported begomoviruses and betasatellites (Akbar et al., 2012). Therefore nothing is known regarding the significance of this recombinant sequence except the possible cause of breakage of resistance of CLCuD.

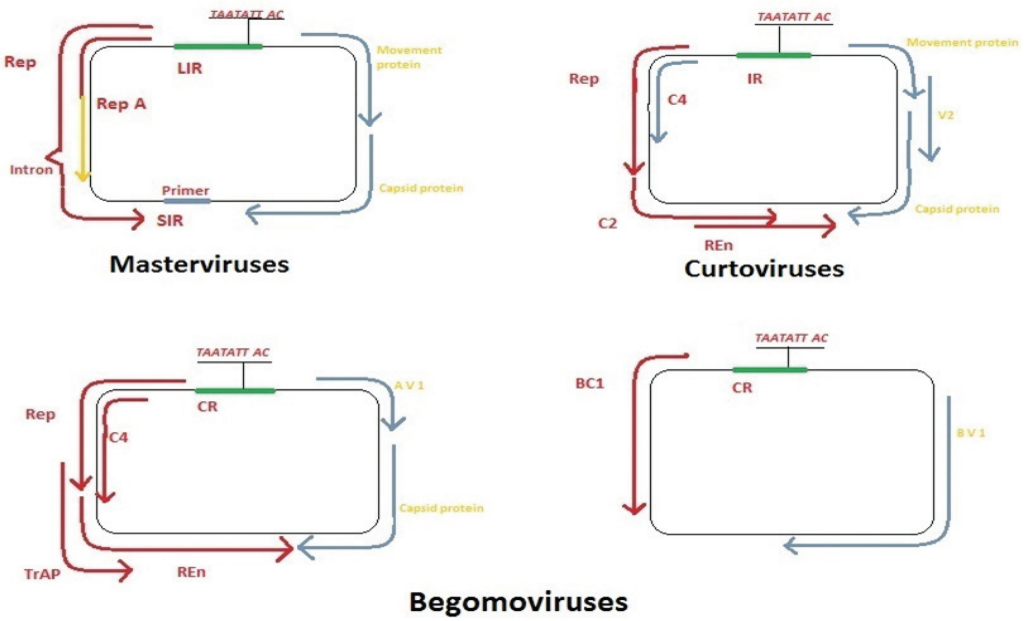

Fig. 1. Genome organization of the three geminivirus genera (Gutierrez C. 1999)

The genetic organization of dcDNA forms is represented. The sequences regulating DNA replications and transcriptional activity are located in the intergenic regions. Mastetrviuses contain a large (LIR) and a small (SIR) intergenic regions, to which a small DNA molecule (primer) is associated (Palmer and Rybicki, 1998; Gutierrez C. 1999). The invariant TAATATT, AC sequence located in the LIR (mastreviruses) and IR (curtoviruses) and CR (begomoviruses) containing the initiation site of rolling-circle DNA replication is shown (Gutierrez C. 1999). Arrows indicate the viral protein which has been named according to either their function, if known or their genetic location and direction of transcription: MP, movement protein: CP capsid protein: Rep, replication protein: TrAP, transcriptional activator, REn, replication enhancer. The RepA protein is also shown in mastreviruses.

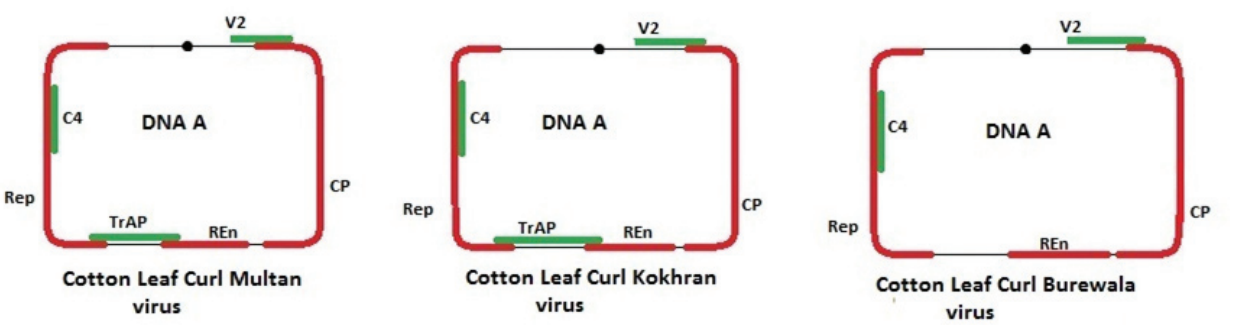

Fig. 2. Diagrammatic comparison of CLCuBuV, a recombinant of CLCuMuV and CLCuKoV

Symptoms

Cotton plants infected by cotton leaf curl virus (CLCuD) show a range of symptoms depending upon the severity of the infection (Farooq et al., 2011). Symptoms of (CLCuD) include curling of leaf, thickening and swelling of veins and production of foliar outgrowth at under side of the leaf, called enations (Mansoor et al., 1997; Harrison et al., 1997). The characteristic symptoms are shown in Figure 3. Two types of vein thickening are produced, major vein thickening and minor vein thickening, thickening starts near the leaf margins and extend inward to form network of thickened major veins (Watkins, 1981). Minor vein thickening occurs as pale green thickening of fine veins of young leaf, this irregular thickening of veins causes the leaf veins to coalesced (Nour and Nour, 1964), CLCuD affects the cotton plant by reducing the internodal distance thus giving cotton plant a stunted growth. Significant Reduction in number of bolls per plant, branches per plant, boll weight, seed cotton yield and deterioration in lint quality occurred due to CLCuD infection (Tanveer and Mirza, 1996).

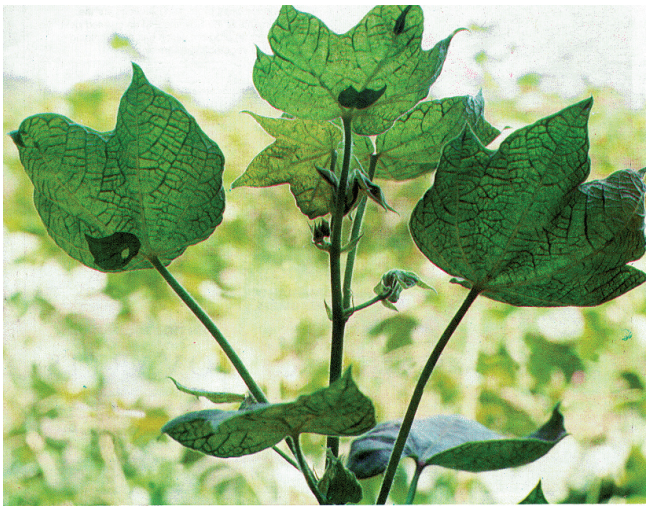

Fig. 3. Typical symptoms (Leaf curling, vein thickening & leaf enation) of CLCuV affected plant

Disease rating scales

For the study of disease severity in field and determining the resistance / tolerance in segregating generation or new genotypes, different methods are used by cotton scientist. The proposed disease rating scale used to determine the level of resistance or susceptibility of cotton lines to CLCuD disease in second meeting of CLCuD in 1996 was decided (Anonymous 1996). Akhtar et al. (2001) and Akhtar (2002) with few modification proposed rating scales for disease reaction and are summarized in Figure 4. By using the scale outlined by Akhtar in (2002) disease intensity and index can be estimated.

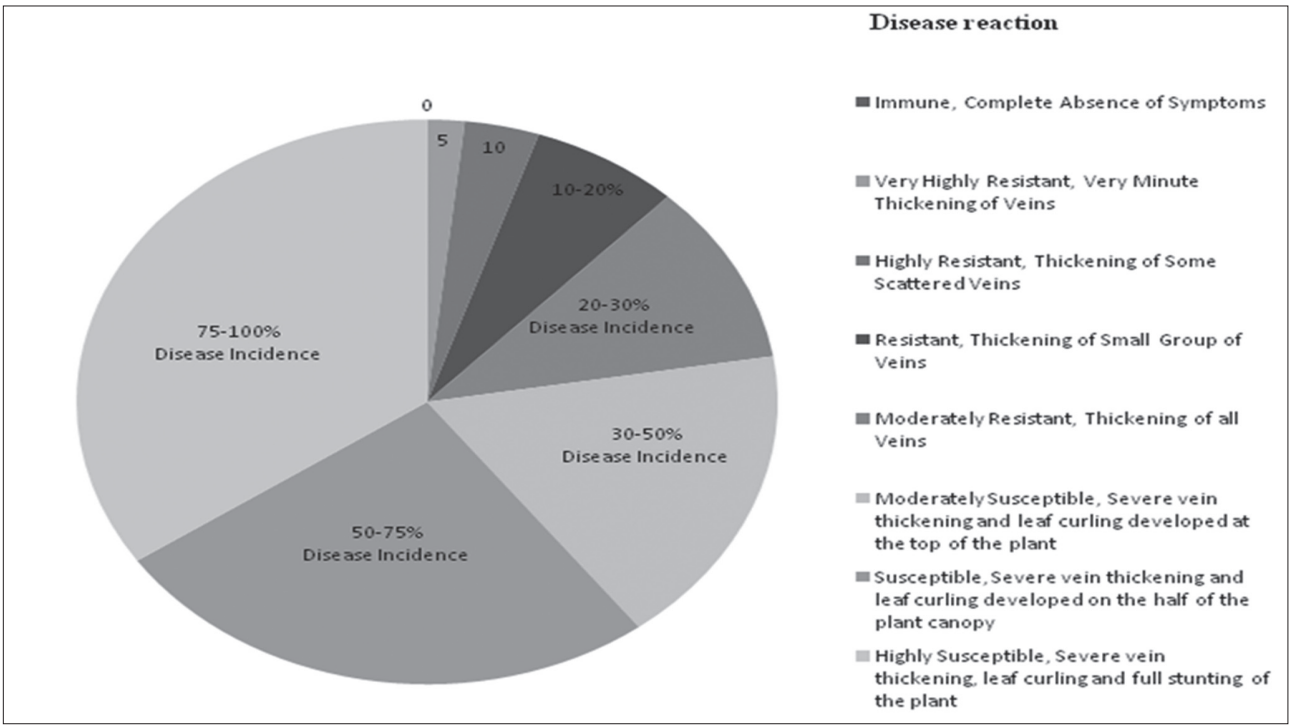

Fig. 4. Compiled disease scales for rating of CLCuD disease

Disease incidence can be calculated as:

Percent disease incidence = sum of all disease ratings of the selected plants / total number of assessed plants×100/6 (Saravanakumar et al., 2007; Anand et al., 2010; Sahi et al., 2007).

Naveed et al. (2007) proposed another formula for calculation of disease incidence:

Percent disease incidence = number of infected plants/total number of plants.

Screening methods for CLCuD

Most commonly used screening methods in field are:

Sick plot technique

Its an easy and very economical method for the phenotypic evaluation of the target varieties and is practiced commonly at various Cotton research stations. In this technique a susceptible genotype S-12, (Anonymous, 2013) is used as spreader in rows between the genotypes to be tested (Shah et al., 2004; Perveen et al*.,* 2005) keept in 1:3 ratios.

Grafting method

In grafting the root stock is the cotton genotype to be tested against CLCuD and scion consisted of the susceptible source of disease inoculums to transmit the disease in stock plants and later on presence of virus is confirmed visually and then by ELISA test (Farooq et al., 2011). This method was used by many scientist (Ali M. 1997; Akhtar et al., 2004, 2010; Shah et al., 2004 and Mansoor et al., 2003a). Three procedure of grafting are mostly applied by the researchers that include bottle graft, top cleft and wedge graft.

Late sowing

New cotton genotypes or segregating population are screened against CLCuD disease by normal and late sowing along with the disease nursery (Khan et al., 2000; Ahuja et al*.,* 2006; Perveen et al*.,* 2010; Iqbal et al*.,* 2011). Iqbal et al. (2010) reported that incidence of CLCuD in late sown cotton (first week of July) reached maximum within 40-50 days after sowing whereas in early sowing (second and third week of April) the CLCuD attack occurs almost 100 days after sowing. So screening of candidate genotypes or segregating material for CLCuD infestation tolerance should be planted in the 1st or 2nd week of July. This method is economically most feasible to screen germplasm, segregating population and candidate varieties against CLCuD tolerance.

Viruliferous whiteflies

In this method of screening cotton germplasm against CLCuD is screened by using viruliferous whiteflies as an inoculation source in net cages on test plants (Mahmood et al., 1994; Monga et al., 2011).

Epidemiology of CLCuD

Climatic conditions like temperature, wind, rainfall, RH (%), light, sex of white flies and plant age affects the incidence and development of cotton leaf curl disease (CLCuD). Bink (1975) suggested that if rainfall occurs just before seedling stage then the population of vector whitefly (*Bemisia tabaci*) will increase due to increase in food sources. Primary sites of infection are established when infected whitefly infects the cotton field (Gusain et al., 1991; Muhammad et al., 1998; Farooq et al., 2011) picks virus from Egg plant, *Abelmoschus esculentus* and *Hibiscus rosa-sinencis* (Briddon and Markham, 2000).

Secondary infection occurs by spread of virus from the primary infection sources through additional vectors that enter in cotton field during whole growing season (Giha and Nour, 1969). Non-significant co-relation was found at minimum air temperature, Relative Humidity at 5 p.m, velocity of wind, sunshine, rainfall and whitefly population on thirteen mutant varieties and negative significant co-relation was found between minimum air temperature and wind velocity (8 a.m) for the development of cotton leaf curl disease (Akhtar et al., 2002b). There is a non- significant relationship of whitefly population with the disease development (Briddon et al., 1998; Hameed et al., 1994; Iqbal, 1993).

Genetic Bases or Inheritance of Resistance to Cotton leaf curl virus (CLCuD)

Tarr (1951) reported resistance to CLCuD as an unstable character. According to Knight (1948) CLCuD is under control of a major gene. Findings of Ali (1999), Rehman et al. (2002) and Haider (2002) suggested that CLCuD is controlled by single gene with dominant effects. Iqbal et al., (2003) reported the involvement of two dominant genes and behaved as dominant epistasis in controlling resistance to CLCuD. Rehman et al*.,* (2005) reported the involvement of three genes in *G. hirsutum* resistance to CLCuD, two for resistance (R_1 CLCuDhir and R_2 CLCuDhir) and a third suppressor of resistance (sCLCuDhir). Quantitative inheritance with predominance of additive gene effects for CLCuD resistance was revealed by Khan et al*.,* (2007). However, earlier the findings of Siddiq, (1970) suggested that a major dominant gene is involved in controlling resistance of CLCuD along with minor (modifier genes).

Although resistant source against this disease (CLCuBuV Burewala) is not available in upland cotton but genetic tolerance can be intensified by gene pyramiding. Two new cotton genotypes MNH 886 and IUB 222 have developed through gene pyramiding which are highly tolerant to CLCuD (Anonymous, 2011b). Genetic pyramiding involved stacking of naturally occurring alleles of tolerant genes into a single elite

genotype in multiple crossing attempts. Monogenic tolerance didn't prove successful for longer period of time and is always at risk in the current world wide viral threat.

Interspecific Hybridization a tool for transferring Cotton Leaf curl virus resistance genes

The available germplasm (exotic and local at all research organizations of Pakistan) of upland cotton is susceptible to CLCuD (Anonymous 2011b). Cultivated species of cotton *Gossypium herbaceum* (A1) and *Gossypium arboreum* (A2) are resistant to this disease (Anonymous 2011a). From genus *Gossypium* eight wild diploid species are found resistant to CLCuD (Anonymous 2011a). These species are *Gossypium anomalum* (B1), *Gossypium capitisviridis* (B3), *Gossypium sticksii* (E1) , *Gossypium somalense* (E2), *Gossypium longicalyx* (F1), *Gossypium gossypioides* (D6), *Gossypium laxum* (D9) and *Gossypium areysianum* (E3).

Gene transfer through conventional breeding from diploid cotton species has rarely been successful due to embryo abortion after fertilization (Ahmad et al*.,* 2011). Cross of diploid species with upland cotton results in sterile F_1 hybrids (Ahmad et al*.,* 2011). To produce hexaploids these sterile hybrids must have to be treated with colchicine (Joshi and Johri, 1972), exogenous hormone application to overcome crossing barriers with *Gossypium* species have been proposed by (Liang and Son, 1982 and Liang et al., 1978).

Inorder to make interspecific crosses successful, between tetraploids *Gossypium hirsutum* L. and diploid *Gossypium arboreum* L. genotypes Gibberalic acid was used to overcome shedding of interspecifically crossed bolls (Mofidabadi, 2009). Boll retention in cross of *Gossypium arboreum* with *Gossypium hirsutum* or in reciprocal cross is very low but F_1 and BC_1 population of this cross were resistant to CLCuD (Ahmad et al., 2011). An autotetraploid of *G. arboreum* L was created and were manually hybridized with allotetraploid *G. Hirsutum* under field conditions and the BC_2 population showed resistance to CLCuD (Ahmad et al., 2011). These findings indicates that using of conventional breeding methods to transfer desirable traits from diploid species is feasible and efforts should continue to transfer the gene resistant to CLCuD from diploid species mentioned above in upland cotton.

At Central Cotton Research Institute Multan, efforts are being carried out to transfer CLCuD resistant gene from above mentioned eight wild and two cultivated diploid species into upland cotton by conventional methods. It is reported that resistance did not remain stable in back cross (upland cotton) and segregating population (Anonymous, 2011a). Results are reported in annual technical progress report 2011 of Cotton Research Station Multan (Anonymous, 2011b).

Conventional precautionary methods

Monga et al. (2001) suggested that off season weeds and hosts are the primary source of spread of cotton leaf curl disease. According to Norula et al. (1999) and Monga et al. (2001) cotton leaf curl disease can be managed and controlled by eradication of plants and weeds that act as a host for cotton leaf curl virus. In early sowing (mid-April to mid-May) severity of disease decreased significantly (Ghazanfar et al., 2007 and Iqbal et al., 2011). Most recommended practices to manage cotton leaf curl disease are to use virus tolerant/resistant cultivars, eradication of causative agents and balanced minerals nutrition (Akhtar et al., 2004).

Biotechnology as a tool to combat CLCuD

Conventional breeding methods have certain limitations because of the sudden changes in climatic conditions and availability of limited resources, with the advancements in biotechnological methods now it is easy to combat cotton leaf curl virus by cloning certain viruses and develop controlling strategies (Farooq et al., 2011). *Gossypium arboreum* L. is free from CLCuD and various other viral and fungal diseases (Briddon and Markham, 2000). *Gossypium arborium* L. has been exploited for isolation of resistant genes and incorporation of resistant genes into susceptible varieties by genetic transformation (Farooq et al., 2011). Molecular markers associated with cotton leaf curl virus disease resistance can enhance the selection efficiency in breeding programmes (Farooq et al., 2011). By using markers, selection for resistance would be easy without being infecting the plants with the pathogen, thereby reducing the chance of pathogen to escape in new environment (Aslam et al., 2000). Aslam et al. (2000) found three DNA marker loci that were linked with each other and had association with cotton leaf curl virus, by evaluating a subset of F_2 plants by selective genotyping with RLFPs. RNA interference is cutting edge technology which can be effectively utilized in the development of resistance against CLCuD (Kasschau and Carrington, 1998; Waterhouse, 2001; Mikhail et al., 2003). Post transcriptional gene silencing is found to be useful for RNA viruses while geminiviruses are effectively controlled by transcriptional as well as post transcriptional gene silencing. Mette et al. (2000) advocated the effectiveness of transcriptional gene silencing against Mung Been Yellow Mosaic Virus. Another much more similar technique of RNAi is DNAi, recently introduced is quiet easy (Hiroko et al., 2004) and cheap method as compared to RNAi. PCR based amplified promoter-less DNA products are enough to cause sequence specific gene silencing in a way like that of RNAi (Voinnet et al., 1998; Palauqui and Balzergue 1999; Rutherford et al., 2004; Hiroko et al., 2004). Hiroko

et al., (2004) devised a DNAi protocol for the functional analysis of Fern *Adiantum*, and silenced several vital genes. This gives an improvisation for the effective utilization of DNAi in the control of CLCuD.

Cotton leaf Curl Virus Management

The ability of the plant to recuperate from the damage caused by cotton leaf curl virus depends upon the balanced used of fertilizers, which in return reduces the chances of damage from cotton leaf curl virus and increases the seed cotton yield (Pervez et al., 2007). Beringer and Tolldenier (1978) and Marschner (1995) reported that plants resistance against diseases can be increased by adequate supply of Potassium (K) because of its functions in osmoregulation, synthesis of molecular compounds and in maintaining energy gradient. Potassium (K) has significant effects on certain disease by its specific role in metabolic function that changes the compatibility relationship of host-parasite (Kafkafi et al., 2001) which advocates its possible utility against CLCuD. Adequate Nitrogen: potassium (N: K) ratio should be maintained, as Nitrogen (N) reduces disease resistance whereas Potassium (K) improves it (Chang and Liang, 1978).

The impact of plant spacing and planting time on yield components of cotton and CLCuD incidence studied and showed that a significant interaction of plant spacing and planting time for seed cotton yield its components and CLCuD incidence. Higher seed cotton yield in early planting with high plant spacing and maximum yield with narrow plant spacing in late planting was observed. The disease incidence and intensity increased in late sowing (Iqbal et al., 2008; Iqbal et al*.,* 2010; Tanveer and Mirza, 1996; James et al*.,* 2004). Iqbal et al. (2008) suggested that cotton genotypes those fell prey to severe incidence of CLCuD can be managed to withstand damage by increasing plant population and nitrogen fertilizer application to achieve optimum seed cotton yield.

Conclusion

In these above mentioned synthetic tetraploids, both diploid species are CLCuD resistant crossed with cultivated one which will probably yield CLCuD resistant inter-specific hybrid. These synthetic tetraploids should be used for introgression of CLCuD resistant gene in *G. hirsutum*. Biotechnological and genetic engineering methods can be used to clone and evaluate different components of viruses and can help scientists to pave out controlling strategies. Unless a CLCuD resistant variety is not developed, highly tolerant varieties should be cultivated and losses due to this disease should minimize by increasing plant population and intensive inputs in late planting.

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