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Review article

A Review about Cotton Leaf Curl Viral Disease and Its Control Strategies in Pakistan

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Abstract

Cotton leaf curl virus (CLCuV) in Pakistan is the most serious threat to cotton crops of last two decades. This diseases causes a huge losses not only to the cotton crops but also the economy of Pakistan is under threat. This problem of Cotton Leaf Curl Disease (CLCuD) is still under discussion among the researchers since it first appeared in 1967 and in 1992-93, it came in epidemic form. The dilemma of CLCuD caused decline in the yield down to 9.05 million bales and 8.04 million bales in 1993-94 in Pakistan. For developing resistant cultivars against the virus to screen against CLCuD, different disease inducing methods such as grafting, delayed sowing and whitefly mediated transfer are used. The epidemiology of diseases is changed by abiotic factors specifically temperature and plant age. Management of CLCuD is the only option that can command the disease in various ways inclusive of change in sowing dates, crop nutrition, cultural practices, vector control, buffer crops and systemic poisoning of cotton seed by seed treatment will make the cotton crop safe in initial 40-50 days after sowing. Biotechnology can also help in controlling this disease through transcriptional gene silencing. By using biotechnological tools broad spectrum resistance can be introduced against all viruses present in the field.

Keywords: CLCuV, *Gossypium hirsutum, Bemisia tabac,* Cotton, Pakistan.

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INTRODUCTION

Cotton (*Gossypium hirsutum L.)* is the greatest central cash crop of Pakistan which belongs to Gossypium Genus and Malvaceae family (Brubaker et al., 1999a). Cotton consists of more than 50 species including wild and cultivated. Fryxell (1979) reported that in nature 45 species are diploid and 5 are allotetraploid. The status of cotton is obvious from the circumstance that it is not only one of most important fiber crops but also it is the second most important oilseed crop of the world (Cherry and Loffler, 1984). Riaz et al. (2013) reported that cotton is grown in warmer climates overall the world. Pakistan has made substantial increase of 11.2 times lint production and 4.4 times seed cotton yield per acre since 1947 but still there is a wide gap of cotton yield between our and other advanced cotton growing counties in the world (Ahmad et al. 2010).

The environmental changes including biotic and abiotic stress are the major threat to food security and agricultural production. Biotic stresses, including viral diseases, are responsible for huge losses in production and quality in all the parts of the world as well as in Pakistan. Cotton leaf curl virus (CLCuV) is furthermost damaging disease which results in enourmous losses (Khan and Ahmad, 2005). Cotton leaf curl virus is the most important cause establishing primary limit (Watkins, 1981; Briddon et al., 2000). This review will help to obtain information about CLCuD and its control through conventional and biotechnological tools.

Background of CLCuV

According to Farquarson (1912) CLCuV was reported for the first time on Gossypium barbadense in Nigeria. The disease occurred suddenly with minor problem including second time epidemic occurrence in 1924 in Nigeria. After 1912, it was recorded in 1924 in Sudan where it was found to be transmitted under natural conditions through grafting and whitefly (Bailey, 1934; Jones and Mason, 1926). Jones and Mason (1926) reported that the disease became a major component in the reducing long stable cotton production in Gezira and Tanzania until 1960. In 1926, CLCuD was reported in Tanzania and in 1959, it was found in the Philippines (Hussain and Ali, 1975). In 1993, the disease was reported for the first time in India on G.hirsutum in Spriganagar area, Rajasthan state (Ajmera, 1994). Rishi and Chauhan (1994) and Singh et al. (1994) reported that CLCuV appeared in 1994 in Haryana and Punjab states on American upland cotton turning into a major threat for the cultivation of cotton in Northern India (Verma et al., 1995). Cotton leaf curl virus is one of the major factors which results a primary limit in cotton production (Watkins, 1981; Briddon et al., 2000). In Pakistan, the disease was first time recorded on few individual plants of Gossypium hirsutum L. at Khokhran which is near the Multan (Hussain and Ali, 1975) and was not such a serious problem in the beginning but with time it became an alarming and potential threat to many of the cotton cultivars (Hussain and Ali, 1975). After 1991-92, it became a serious danger for cotton production until 1996 when resistant variety of cotton against CLCuV was developed (Ahmad et al., 2010). Mansoor et al., (2003) reported that during 2001

in Burewala CLCuD arose again breaking resistance which was present in all available lines of cotton. Now this became a destructive disease of cotton in Punjab area of Pakistan. Due to almost the same symptoms of Burewala virus to previous CLCuV, this virus is only 8% different from previous virus which was examined on molecular basis (Mansoor et al., 2003).

Symptoms

The symptoms of plants which are infected by CLCuD may vary depending on the disease severity (Farooq et al., 2011). These authors reported that the symptoms of CLCuD typically are yellowing and thickening of the smaller veins that are present on the lower surface of the younger leaves (Fig. 1).

Figure 1. Cotton leaf curl virus

There are two kinds of vein thickening - first small vein thickening and second main vein thickening (Ahmad et al., 2010). Mansoor et al. (1993) and Nateshan et al. (1996) reported that the initial symptoms of CLCuD contained temporary vein clearing on the young leaves. Leaves curl upward or downward with plant growth (Fig. 2a), stunted under the severe attack of the disease due to reduction of the distance between nodes (Briddon et al., 2001; Qazi et al., 2007). Infected leaves also produce a cup shape outgrowth on the lower side of the curled leaves that is named Enation (Fig 2b) (Mansoor et al., 1993; Harrison et al., 1997).

Figure 2. Typical symptoms (leaf curling, vein thickening and leaf enation) of CLCuV affected plant

Rehman et al. (2000) and Monga et al. (2011) reported that at the seedling stage, the appearance of this disease was so serious that retarded flowering, boll formation, maturation and dramatically reduced the fiber quality and cotton seed yield.

Epidemiology

Different climatic factors such as temperature, wind and rainfall are involved in the development of CLCuD (Farooq et al., 2011). As stated by Blink (1975) seedling before rainfall may be posed an increased population of vector due to abundance supply of food source. Ahmed et al. (2013) showed that significant correlation exists between temperature and CLCuD and between PAN evaporation and CLCuV during July in Multan district of Pakistan which is regarded to be the hot spot area for the development of this disease. Farooq et al. (2014) reported that cotton which is grown only for a part of the year, cultivated host and alternate weeds function as reservoirs for the virus.

Primary sites of infections and fields in which cotton is infected by whitefly are established (Farooq et al., 2014). These sites as well as additional vectors serve as secondary spread of the CLCuD to other plants which during the whole growing season enter in the field (Giha and Nour, 1969). Khan et al. (1998) studied the relationships between air temperature (maximum and minimum), relative humidity, wind movement and rainfall and the percentage of plants which are infected by CLCuD in 80 cotton varieties using regression. The rate of infestation of the disease increased in the range of minimum and maximum temperatures of 25-30^{o}C and 33-45^{o}C respectively. The authors also showed non-significant correlation between the intensity of CLCuD and the population of whitefly, and also poor correlation between the humidity and the weekly rainfall and the development of the disease. Akhtar et al. (2002b) reported that there was non-significant correlation between the relative humidity (measured at 5p. m.), wind velocity, sunshine, maximum weekly air temperature and the population of whitefly in 13 cotton varieties. Furthermore, they showed that there was a negative correlation for the development of CLCuV disease between the velocity of wind (at 8 a. m.) and the minimum temperature of the air, however, positive correlation that existed between the plant age and percentage of the disease incidence

was found (Akhtar et al., 2002b). Maximum percentage of disease index that was recorded at 6 weeks old seedlings gradually decreased with the age of the plant (Farooq et al., 2014). As stated by Briddon et al. (1998), many researchers observed non-significant relationship present between CLCuD and the population of whitefly.

Genetic Bases or Inheritance of Resistance to CLCuD

In a study from 1951, Tarr showed that the resistance expressed to CLCuD has unstable character. According to Knight (1948) CLCuD is controlled through a major gene. There are two dominant genes that control the development of CLCuD in upland cotton (Randhawa, 1999). Hutchinson and Knight (1950) reported that breeding for development of resistance against CLCuD has been acquired through the involvement of a combination of minor genes using recurrent selection. Resistance which depends on major genes (dominant genes) against CLCuD may no longer exist and disappear quickly due to the evolution of the pathogen occurred for these genes (Azhar et al., 2010). According to studies of Ali (1999), Rehman et al. (2002) and Haider (2002), it was suggested that CLCuD is under control of single genes having dominant effects. Two dominant genes control CLCuD that behaved as dominant epistasis in controlling resistance against CLCuD (Iqbal et al., 2003) and there are three genes involved in resistance to CLCuD in upland cotton (G.hirsutum) (Rehman et al., 2005). It was also found that two out of three genes, function as resistance to CLCuD (R1 CLCuD hir and R2 CLCuD hir) while the third gene that is suppressor gene serves to suppress resistance (sCLCuD hir) (Rehman et al., 2005). Ahuja et al. (2006) reported that there are two genes involved in the resistance to CLCuD with duplicate dominant, dominant inhibitory and duplicate recessive non-allelic interaction (epistasis) and three genes with triplicate dominant epistasis. Further, Khan et al. (2007) stated that the inheritance of resistance for CLCuD is not known, and it is still under discussion whether it is nuclear (nucleus) or extra-nuclear (cytoplasm), however, both report that maternal effects are present. According to Khan et al. (2007) there is quantitative inheritance for the resistance against CLCuD with predominance of additive gene effects. In earlier the studies though (Siddiq, 1970) major gene functions in controlling the resistance of CLCuD along with another minor gene (modifier genes) was observed. Since the resistance source against CLCuBuV (Cotton leaf curl burewala virus) is not present in American upland cotton but through gene pyramiding, genetic tolerance can be intensified (Iqba et al., 2014). Two new cotton genotypes IUB222 and MNH 886 have been developed against this disease by gene pyramiding which exhibit high tolerance (Anonymous, 2011b). According to Aslam et al. (2000), a cross made between Gossiupium barbadense L. (Giza-45) and Gossiupium hirsutum L. (Reba P-288) determined the presence of effect of a single dominant gene. The F1 of the crosses made between highly susceptible S-12 and highly resistant LRA-5166 varieties had all plants free from the virus and their F2 was found close to 1:3 ratio which indicated presence of single gene which had inheritance of the resistance towards

CLCuD (Mehmood, 2004; Rehman et al., 2005). In the same cross (LRA-5166 x S-12), there was no single gene with major effect responsible for CLCuD (Khan et al., 2007).

Screening Methods for CLCuD

Many screening methods are applied for CLCuD. The most common ones used in the field are described below.

Sick Plot Technique

It is an easy method that is used for the phenotypic evaluation of the target varieties and is practiced commonly at variously Cotton research station (CRS) (Iqbal et al., 2014). A susceptible genotype S-12 is used in this technique (Anonymous, 2013) functioning as spreader in rows between the genotypes to be tested keep in 1:3 ratios (Shah et al., 2004; Perveen et al., 2005).

Grafting Method

This method has been used by many scientist (Ali M. 1997; Mansoor et al., 2003a; Akhtar et al., 2004; Shah et al., 2004). In this method the root stock of the cotton genotype which is tested against CLCuD and scion contained the susceptible source of disease inoculums that are used to transmit the disease in stock plants, later on the presence of virus is confirmed visually and then by ELISA test (Farooq et al., 2011). Bottle graft, top cleft and wedge graft are three procedures of grafting that are mostly used by the researchers in practice.

Late Sowing

New genotypes of cotton or segregating population in F2 are screened against CLCuD by normal and late sowing including with the disease nursery (Khan et al., 2000; Ahuja et al., 2006; Perveen et al., 2010; Iqbal et al., 2011). The occurrence of CLCuD reached maximum within 40-50 days after sowing in late sown cotton (first week of July) while in early sowing (second and third week of April) the attack of the CLCuD occurs almost 100 days after sowing (Iqbal et al. 2010). Therefore, screening of candidate genotypes or segregating material for tolerance against infestation of CLCuD should be panted in the 1st or 2nd week of July so this method is economically most feasible to screen germplasm, segregating population and candidate varieties against CLCuD tolerance.

Viruliferous Whiteflies

Mahmood et al. (1994) and Monga et al. (2011) reported this method of screening of cotton germplasm against cotton leaf curl disease to be very useful. Cotton germplasm is screened by using viruliferous whiteflies that are used as an inoculation source in net cages on test plants.

Control Measures (Non-Biotechnological Tools) and Recommendations

The development of tolerant varieties against disease is so far one of the solutions but when resistance sources become inadequate then management of the disease is quite appropriate (Farooq et al., 2011)**.** In cotton, host plant resistance is a long term and explored strategy that is used to protect plants from the attacks of CLCuD (Jones, 2001; Solomon-Blackburn and Bradshaw, 2007).

Monga et al., (2001) reported the primary inoculum existing in the form of weeds during off season and other hosts to be the major source to spread of CLCuD. The control of the vector whitefly and the eradication of weeds contributing to the hospitality of CLCuV are some of the strategies that are used in the management of CLCuD (Narula et al., 1999; Monga et al., 2001). The plants escape the most susceptible stage by using insecticides even if infection occurs at later stage and the severity of losses may be avoided when symptom will begin to appear after 65-90 days (Singh et al., 2002; Monga et al., 2011).

Numerous agronomic practices such as sowing time and application of nutrients – nitrogen (N) and potassium (K) can cause severe disease problems as choosing best sowing time for specific variety in different region is difficult so that too early and too late sowing may cause problem of disease and pests (Farooq et al., 2011). According to Ghazanfar et al., (2007) the suitable time of sowing preferably mid April to mid May results in decrease of the occurrence of disease as compared to late sowing from mid May to June. If the distance between plants is increased in early sowing and decreased under late sowing condition, then it is effective in the management of CLCuD (Iqbal and Khan, 2010). The authors also determined that the infestation of CLCuD reached its peak after 105 days of sowing while in the case of late sown crop (15th June or late), the infestation becomes severe after 45 days of sowing. Hence they suggested 15 cm plant spacing that is appropriate to manage CLCuD in the case of planting later than 15 th of June.

The appropriate nitrogen concentration in the case of susceptible cultivars plays a vital role to tackle the severity of the disease while not affecting the resistant cultivar (Farooq et al., 2011). Strategies can be planned to prevent, escape, avoid and control viral disease by understanding physiological basis of nutrition (N) (Zafar et al., 2010).

The virus resistant cultivars, management of causative agents and mineral nutrition are the most recommended management practices that are used to handle CLCuD disease (Akhtar et al., 2004). Kafkafi et al. (2001) showed effect of K application on the disease through specific metabolic functions change relationship of host-parasite environment. After experiments conducted on the role of K in the control of CLCuD, Pervez et al. (2007) concluded that the application of K up to 250 kg/ha resulted in the reduction of disease which is about 12%- 38%. The higher K levels led to considerable increase in the cotton seed yield increased (up to 37% when compared to zero-K).

Beringer and Tolldenier (1978) and Marschner (1995) studied that resistance in plants can be increased against diseases by adequate K supply as its functions in osmoregulation, synthesis of molecular compounds and in maintaining energy gradient of energy. Therefore adequate N:K ratio is very important that should be maintained because nitrogen reduces disease resistance while potassium improves it (Chang and Liang, 1978).

Recent Advances to Combat CLCuV through Biotechnological Tools

Due to the abrupt changes in climatic conditions and the accessibility of limited resources, conventional breeding methods are not successful and have certain limitations. However, nowadays it is easy to combat CLCuV by cloning certain virus due to advancement in biotechnological methods and develop controlling strategies (Farooq et al., 2011).

As stated by Agrios (1997), during domestication of plants from wild to cultivated forms, diseases have caused a huge loss in yield.

The main problem that plant breeder has to face is the introgression of resistance traits controlling genes for development of resistance in plants through using conventional procedure of breeding (Farooq et al., 2011). Nowadays, the crop plants may have resistance against certain disease that is developed by genetic engineering and this resistance against certain pathogens is controlled by single or multiple genes (Crute and Pink, 1996). Pathogen disease resistance (PDR) methodology by RNA mediated technology (sense and anti-sense RNA mediated) and protein mediated resistance has been recognized to combat different viruses due to the lack of natural resistance against diseases in plants. Many genes have been integrated in a number of plants which are used to engineer PDR, especially in those crops in which natural resistance genes are not found (Gallitelli and Accotto, 2001).

American upland cotton (*Gossipium hirsutum* L.) is free from CLCuD and various other viral and fungal diseases (Briddon and Markham, 2001). Through genetic transformation approach, resistant genes are isolated and incorporated into susceptible varieties (Farooq et al., 2011). Molecular markers that are related to CLCuD resistance can improve the efficiency of selection in the breeding programmes (Farooq et al., 2011).

Aslam et al. (2000) reported that the use of markers in the selection for resistance would be easy without infecting the plants with pathogen, thus reducing chance of pathogen to escape in new environment. The researchers found three DNA marker loci that had association with CLCu V and were linked with each other by evaluating a subgroup of F2 plants by selective genotyping with the use of RFLPSs.

RNA interfering is cutting edge technology which can be used efficiently 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 virus while geminiviruses are

effectively controlled by transcriptional as well as post transcriptional gene silencing. Mette et al. (2000) recommended the effectiveness role of transcriptional gene silencing against Mung Been Yellow Mosaic virus. DNAi which is new recently introduced technique is quite easy and cheap method as compared to RNAi although it is much more similar to RNAi technique (Hiroko et al., 2004).

Promoter-less DNA products which are amplified by PCR are enough to cause sequence specific gene silencing in a similar 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) proposed a DNAi protocol that is utilized for functional analysis of Fern Adiantum and silenced numerous vital genes. This gives an improvisation to control CLCuD by using DNAi effectively.

Hashmi et al. (2011) reported two truncated forms of replicase (tACI) gene were introduced into *Gossipium hirsutum* through cloning by exploiting transcriptional control which are capable to express only N-terminal 669bp (5'ACLI) and C-Terminal 783bp (3'ACI) nucleotides. Interference technology is used to impair cotton leaf curl virus in transgenic cotton by using a strain LBA 4404 of *Agrobacterium tumefaciens.*

Transformed plants confer resistance by inhibition of viral genome and satellite DNA components due to the over expression of either above discussed nucleotides when they were compared with control non-transformed plants.

Northern blot hybridization in early and late growth stages showed high transgenic expression (Hashmi et al., 2011).

Other methodology that is used to develop resistance is isolation and integration of resistance genes present in related plant species for certain virus. These resistance genes are needed to be incorporated into the commercial varieties that are available in different genotypes for efficient management of disease (Kumaran, 2005).

According to Azhar et al. (2010b), the wild species of Gossipium especially *G.thurberii*, *G.anomalum, G.raimondii*, *G.armourianum* and *G.tomentosum* are a good source of resistance against different insect pests such as bollworm, jassids, whitefly including disease such as bacterial blight and Verticillium wilt. Although genetic variation found in *G.hirsutum* is scanty yet it has ability to resist against sucking pests such as white flies, thrips, leafhoppers and aphids (Rahman et al., 2007; Kantartzi et al., 2008).

Effect of CLCuD on the Yield and Fiber Traits

In textile industry, cotton fiber (lint) is the most important product and CLCuD also affects the quality traits of fiber (Ali et al., 1995; Khan et al., 2000; Khan et al., 2001; Kalhoro et al., 2002; Khan et al., 2003). The disease has not only effect on yield but also deteriorates the quality traits of fiber like

ginning turnout percentage, staple length, fiber uniformity index, fiber bundle strength, fiber fineness and maturity ratio. Due to change in composition of the major components of fiber such as cellulose, protein, wax and pectin, the quality of fiber is negatively affected (reviewed by Farooq et al., 2011; Iqbal and Khan, 2010). The losses that occur due to CLCuD depend on the infectivity as well as the variety. Akhtar et al. (2003b) reported pronounced damage of CLCuD appeared at early stages but at later stages there were only minor infestations. The damage due to CLCuD differs on various parts of plant which ultimately results in reduction of the yield. It can reduce various yield contributing traits like fiber length 3.44%, fiber strength 10%, elongation percentage up to 10% boll weight 33.8%, 73.5% in boll per plant, GOT % up to 3.93%, seed index 17.0% and yield per plant 64.5% (Ahmed, 1999). But according to Idris (1990), the virus has significant impact on the yield but not on the fiber quality. Losses in production of cotton that occurred due to CLCuV during last 20 years are given below in Table 1.

Table 1. Losses to area (1000) hectares and production (1000) bales of cotton due to CLCuV in Pakistan in the last 20 years.

Year	Partial losses	Complete losses	Total area affected (1000) hectares	Losses (1000) bales
1988-89	-	0.06	0.06	0.3
1989-90	-	0.2	0.2	1
1990-91	-	0.8	0.8	4
1991-92	11.3	2.8	14	20
1992-93	364	121	485	750
1993-94	607	282	889	1880
1994-95	407	-	407	221
1995-96	882	-	882	447
1996-97	1623.9	137.4	1761	2100
1997-98	762.9	19.5	782	1118.1
1998-99	457.9	-	458	587.1
1999-00	289.1	-	289	370.5
2000-01	90.1	-	90	111.2
2001-02	66.6	-	67	82.3
2002-03	357.7	2.15	359	265
2003-04	488.7	14.1	503	503.9
2004-05	127.8	31.1	130	967.1
2006-07	1686	25.21	1712	1231.7
2007-08	1432.8	2.5	1435	953.5
2008-09	1440.1	40.25	1480	1115.7

Conclusion

All the above mentioned measures that are used to control CLCuD depend on the conditions. Development of resistant varieties including good agronomic practices, fertilizer, insecticidal control

and biotechnological methods can be used alone or in combination to control this severe disease which is still a challenge even after twenty years of extensive research. Unless a CLCuD resistant variety is not developed, highly tolerant varieties should be cultivated and losses due to this disease should be minimized by increasing plant population and intensive inputs in late planting.

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