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Cotton leaf curl virus disease a principle cause of decline in cotton productivity in Pakistan (a mini review)

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Abstract

Among most serious threats of last two decades with reference to cotton crop in Pakistan is Cotton leaf Curl Virus. This disease inflicted huge losses not only to the cotton crop but also to the economy of Pakistan. It first appeared in 1967 on few plants in Multan and in 1992-93, caused a decline in production down to 9.05 million bales and during 1993-94, to 8.04 million bales well below the estimated targets in Pakistan. Various disease inducing procedures like grafting, delayed sowing, whitefly mediated disease transfer were exploited to screen out material against this notorious disease. Climate change and weather fluctuations have a profound influence on the spread of Cotton Leaf Curl Virus. Climate change is altering temperature and precipitation patterns, resulting in the shift of some insect/pest from small population to large population thus effecting crops yield. The abiotic factors like temperature and plant age influence cotton leaf curl virus disease epidemiology. Fiber quality traits like Ginning out turn, fiber length, fiber uniformity index, fiber fineness, fiber bundle strength, maturity ratio also deteriorated because of change in composition of major fiber components including cellulose, protein, wax and pectin. The ambiguity about inheritance is also prevailing whether it could be through dominant or recessive genes which may be monogenic or polygenic whereas Extrachrmosomal inheritance is also under discussion. The resistance break down depends upon the evolutionary potential of the pathogen and possibility of recombinations, by which new variants of viruses evolved. The resistance gained for Multan-CLCuV became susceptible to Burewala-CLCuV due to virus mutation and lack of durable resistance. Virus tolerant cultivars along with management practices like changing sowing dates, proper crop nutrition, better cultural practices, efficient vector control and buffer crops are the ways that can help to get better crop. Systemic poisoning of cotton seed by seed treatment may make the cotton crop safe in initial 40-50days after sowing. Biotechnology can also help in controlling this disease through transcriptional gene silencing. By exploiting biotechnological tools broad spectrum resistance can be introduced against all viruses present in the field.

Keywords: Epidemiology, Inheritance, Polygenic, Pakistan, Gene silencing, Extrachrmosomal

The importance of cotton is evident from the fact that it is not only the most important fiber crop but also the second most important oilseed crop of the world (Cherry and Leffler, 1984). In Pakistan Cotton (*Gossypium hirsutum* L.) is grown in warmer climates throughout the country (Riaz *et al.* 2013). In previous twenty years Cotton leaf curl virus disease has become a serious threat to cotton productivity in Pakistan. Sustainable cotton production requires identification and cultivation of stable cultivars Farooq *et al.* 2013. The yield sustainability in cotton still not attains because of several causes but CLCuD is notorious one. Cotton leaf curl virus has interesting evolutionary story. It was reported first in Nigeria (1912) on *Gossypium peruvianum* and *Gossypium vitifolia*, Sudan (1924), Tanzania (1926), Philippine (1959) but in Pakistan CLCuD was first recorded in the 1967 in Multan district on scattered *hirsutum* plants (Hussain and Ali, 1975). It was not well thought-out as a serious disease up to 1987 but appeared in epidemic form in 1992-1993 when 1.3 million bales of cotton were lost over an area of 24.28 hectares. The financial losses with the estimated value of $5 billion (US) to the nation occurred from 1992-1997 (Briddon and Markham., 2001). In 1997, CLCuD was reported from Sindh province of Pakistan which was previously free from this disease (Mansoor *et al.*, 1998). It is very complicated to calculate the precise estimates because the occurrence of CLCuD varies from year to year and also varies from area to area under cotton cultivation.

Symptoms

Cotton leaf curl disease (CLCuD) infected plants may show a wide range of symptoms depending on the severity of disease. Typical symptoms include thickening and yellowing of small veins on the lower surface of young leave. Under severe attack of disease leaves curl downward or upward and plant growth stunted due to reduction of inter-nodal distance and in some cases an outgrowth called enation appears on lower side of curled leaves (Qazi *et al.*, 2007). Cotton plant infected with CLCuD showing veins thickening and yellowing, upward curling (Fig. 1), down ward curling (Fig. 2), enations (Fig. 3) on the underside of the leaves, Stunting of cotton plant (Fig. 4).

Figure 1. Upward curling along with thickening of leaves of cotton plant.

Fig 2. Severe curling along with thickening of leaves of cotton plant.

Figure 3. Enations on the underside of Cotton leaf.

Figure 4. Stunting of the cotton plant.

Chasing symptoms

The attack of sucking pests (Jassid and Whitefly) on cotton (*Gossypium hirsutum* L.) and their symptoms resemblance with cotton leaf curl virus disease create a difficulty by virtue of assessment among the farmers and researchers at early stages of plant development. The cotton leaf curl virus disease (CLCuD) symptoms coincidence with attack of Jassid (*Empoasca facialis*) that suck cell sap under side of leaves by which leave render down ward curling, turn yellowish to brown, retard fruiting capacity, sever incidence results stunting growth of young plants, reduce seed cotton yield and fiber quality. Whitefly (*Bemisia tabaci*) is also sucking pest that causes similar infection like Jassid but with an extra responsibility of virus transmitting vehicle.

As the symptoms are identified the percent asperity and range of rating scales of symptoms are still under discussion. Keeping in view the severity of disease the rating scales for viral diseases are also used other than cotton crop like tomato, Chilli, Cucumber and Tobacco with the range of zero to six numerically, where these scale give the symptom description also determined the level of resistance or susceptibility. If we keenly observe the scale 2 and 3 with reference to moderately resistance and moderately susceptible both are vice versa but used separately. The disease scale that has been generally used in cotton is based on Severity Index (SI) and Percent Disease Index (PDI %). The formula used to calculate both of these two parameters has been reported by Akhtar *et al.* 2003, 2010 and given in Table 1.

Table 1. Disease scale proposed by Akhtar *et al.* 2010

Symptoms	Disease index%	Rating	Disease Response
Complete absence of symptoms	0	0	Immune
Thickening of few small scattered veins or only presence of leaf enations on one or few leaves of a plant observed after careful observations.	0.1–10	1	Highly resistant
Thickening of small group of veins, no leaf curling, no reduction in leaf size and boll setting.	10.1-20	2	Resistant
Thickening of all veins, minor leaf curling & deformity of internode with minor reduction in leaf size but no reduction in boll setting.	20.1-30	3	Moderately resistant
Severe vein thickening, moderate leaf curling followed by minor deformity of internodes and minor reduction in leaf size and boll setting.	30.1-40	4	Moderately susceptible
Severe vein thickening, moderate leaf curling & deformity of internodes with moderate reduction in leaf size and boll setting followed by moderate stunting.	40.1-50	5	Susceptible
Severe vein thickening, leaf curling, reduction in leaf size, deformed internodes and stunting of the plant with no or few boll setting.	>50	6	Highly susceptible

Foliar outgrowths (enation) will be marked with ‘‘E’’ where observed. (This rating scale is proposed by Akhtar *et al.* 2010 in cotton).

Whitefly and virus

A notorious group of viruses belongs to genus Begomovirus cause major threat to cotton crop, which is well known as Cotton Leaf Curl Virus disease (CLCuD) and is transmitted by whitefly i.e. *Bemisia tabaci* complex (including *B. argentifolii*) in persistent manner (Rybicki and Fauquet, 1998). Most of the begomoviruses comprised of two genomic components called DNA-A and DNA-B, which are indispensable for disease that is transmitted by whitefly *Bemisia tabaci* (Monga *et al.* 2011).

Cotton whitefly reported as pest of Tobbaco over 100 years ago (Anonymous, 1993). It is the most vital sucking pest of both industrial and food crops like Cotton, Sunflower, Melon, tomato, Brinjal etc. (Rafiq *et al.,* 2008). Its polyphagous nature is confirmed over 500 plant species all over the world including Asia, Africa, America, Europe, Russia, Australia and Pacific Islands (Greathead, 1986). In cotton growing areas of Central Punjab it has been reported in about 164 plant species (Attique *et al.,* 2003). In 16 of the 27 cotton growing countries whitefly is recognized as a major pest during mid to late sowing time.

Conditions suitable for the spread of disease

Climatic conditions like rainfall, wind and temperature have an influence in the spread of CLCuD in Africa. Rainfall prior to seedling may result in the development of increased population of vector due to abundance in food source (Bink, 1975). Ahmed et al .2013 found significant correlation between CLCuD and temperature and between CLCV and PAN evaporation during the month of July in Multan district of Pakistan which is considered to be the hot spot area for this disease.

As cotton is grown only for part of the year cultivated hosts and alternate weeds serve as virus reservoirs. Whitefly infects cotton fields and primary sites of infections established. Secondary spread to other plants may occur from the primary sites and from additional vector which enter the field during the whole growing season (Giha and Nour, 1969). Khan *et al.* (1998) used regression analysis on weekly air temperature (Maximum and Minimum), rainfall, relative humidity and wind movement relationship with % plant infection by CLCuD on eight varieties of cotton. Disease infestation increased in the range of Maximum and Minimum temperatures 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 intensity and whitefly population on all varieties studied. Akhtar *et al.*, 2002b found non significant correlation of weekly maximum air temperature (°C), % relative humidity (5 p.m.), wind velocity, rainfall, sunshine and white fly population on thirteen mutant/varieties and negative significant correlation between minimum air temperature and wind velocity (8 a.m.) for CLCuV disease development. They also found positive and significant correlation between % disease incidence and plant age. Maximum disease index % was recorded at 6 week old seedlings and it gradually decreased with increase in age of plant. Many researchers found non significant relationship of white fly population with disease (Briddon *et al.*, 1998).

Effect of CLCuD on yield and fiber traits

Losses due to CLCuD are dependant on infectivity time and variety. The pronounced damage of CLCuD is at early stages but at later stages results minor infections (Akhtar *et al.*, 2003). CLCuD damage differs on various plant parts and ultimately results in reduction of yield. It can reduce boll weight 33.8%, 73.5% in bolls per plant, GOT% upto 3.93%, seed index 17.0% and yield per plant 64.5% (Ahmed, 1999). Production losses due to CLCuV during last 20 years are given in Table 2. The cotton fiber (lint) is the most important commodity for textile industry and CLCuD also affects fiber quality traits (Kalhoro *et al.*, 2002). According to Ahmed *et al.*, 1999 CLCuD can decrease fiber length 3.44%, fiber strength 10% and elongation percentage upto 10%. Akhtar *et al.* 2009 studied impact of CLCuD on fiber quality traits and the findings depicts that the CLCuD significantly affect traits like GOT, fiber length, fiber uniformity index, short fiber index, fiber fineness, fiber bundle strength yellowness and maturity ratio. In their studies they observed significant affects of this viral disease on cellulose, protein, wax and pectin which are the major constituent of fiber. But in view of Idris (1990) virus has significant impact on yield but not on fiber quality.

Table 2. Losses to area (1000) hectares and production (1000) bales of cotton due to CLCuV in Pakistan in 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

Resistance inheritance

When the actual designation of disease is realized about its symptoms, vector (whitefly), transmission and environmental conditions then the next step is to solve the problem only on genetic basis rather than the Entomological, Pathological and Agronomical schools of thoughts, which are also important. As the inheritance of resistant genes for disease is still under discussion whether it is nuclear (nucleus) or extra-nuclear (cytoplasm) but both reports about maternal effects are available (Khan *et al.*, 2007). The breeding for cotton leaf curl disease (CLCuD) resistance has been achieved through assemblage of minor genes by recurrent selection (Hutchinson and Knight, 1950) and similar sayings by Azhar *et al.*, (2010) that the resistance depends on major genes (dominant genes) may lost quickly because of evolution of pathogen for these genes. An alternative approach is needed for partial resistance that depends on the recombination of minor genes (recessive genes).

Resistance breakdown

The secret of inheritance is still under discussion and another idea of resistance breakdown was initiated that so called achieved resistance has been broken down by virus mutation whereas the symptoms and parameters for identification are still same in practice. García and McDonald, 2003 reported that the virus mutation requires 25 years at least then who does it possible after 1967s our researchers get early resistance and instantly destroyed the integrity. Whereas the changing climate scenario, cotton varieties either susceptible are sown early can escape from virus and whitefly but the resistant one could be susceptible in late sowing which is the cause of ambiguity between susceptible and resistant.

The concept of polygenic mode of inheritance of cotton leaf curl disease was changed into single dominant gene (with minor modifier genes) as determined by Saddig (1968) and also clarified by Ahuja *et al.* (2007). The cross between Gossypium barbadense L. (Giza-45) and *Gossypium hirsutum* L. (Reba P-288) determined the effects of single dominant gene supported by Aslam *et al.* (2000). The F1 of crosses between highly susceptible S-12, highly resistant LRA-5166 varieties were found all virus free plants and their F2 was close to 1:3 ratios which exhibit the presence of single gene for the inheritance of resistance against CLCuD reported by Rehman *et al.* (2005), Whereas in same cross (LRA-5166 × S-12) no single gene of major effect found to be responsible for cotton leaf curl disease (Khan *et al.*, 2007).

Resistance sources

The wild species of *Gossypium* are potential source of resistance to biotic (insect and diseases) and abiotic (salinity, cold, drought, heat) stresses. *G. anomalum*, *G. longicalyx*, *G. stocksii*, *G, raimondii* and G. *sturtianum* have a source for the improvement of fiber quality characters whereas *G. thurberri*, *G. anomalum*, *G. raimondii*, *G. armourianum* and *G. tomentosum* are the best sources for resistance of insect pests including whitefly which is the main vector for the inoculation of CLCuD (Azhar *et al.*, 2010).

Screening methods employed to develop CLCuD tolerant materials

Screening methods those commonly used is the exploitation of virus spreader line (S-12) and white fly as a source of transmission vector Shah *et al.* 2004 and Perveen *et al.* 2005. For transmission through spreader line these researcher used S-12 the popular and most susceptible variety to CLCuD disease. This variety was planted in rows among the tested genotypes for natural spreader of disease. Shah *et al.* 2004 proposed whitefly mediated transmission using insect proof cages. Another method that was used for screening is the sowing time difference i.e. normal and late sowing along with disease nursery (Ahuja *et al.*, 2007; Perveen *et al.*, 2010). They established CLCuD nursery near the experimental area to allow the spread of whitefly vector throughout the season and tested different sowing dates.

Grafting is the most efficient method to transmit the causal agent as grafted plants develop symptoms within 14-30 days depending upon varietal susceptibility/resistance (Akhtar *et al.* 2001; 2002b). Grafting as a successful method to inoculate CLCuD was used by Akhtar *et al.* 2002 (a, b, c), 2004, 2010 and Shah *et al.* 2004. For grafting researchers employed three procedures like Bottle graft, top cleft and wedge graft. In this procedure the stock used as resistant and scion as susceptible source for inoculation of disease and later presence of virus was confirmed by the use of ELISA.

PCR can be used as a reliable tool for the detection of viruses. As the geminiviruses are small, single stranded and have circular genome thus PCR can be efficiently used for their detection. Several degenerate primers have been designed for the detection of these viruses (Briddon and Markham 1994). With the help of these primers previously uncharacterized geminiviruses can be amplified, and primers designed on the basis of non-conserved sequence can be exploited to detect a particular virus and strain of that virus (McGovern *et al.*, 1994).

Another method to screen the germplasm against cotton leaf curl virus is through inoculation using veruliferous whiteflies in net house conditions either by open choice method or through release of counted veruliferous flies on test plants under plastic jars in polyhouse for fixed interval (Monga *et al.*, 2011)

Pollen irradiation technique may be used as a criterion to develop CLCuV tolerant material for creating genetic variability in cotton germplasm. Aslam and Elhai 2000 used pollen irradiation technique. They attempted different crosses as reported earlier by Doak, (1934) by applying irradiation doses i.e., 5-10Gy (Aslam and Stelly, 1994) to create more genetic variability.

Control measures (non-biotechnological tools) and recommendations

Though the solution of various diseases is the development of disease tolerant varieties but disease management is quite appropriate when resistance sources are inadequate. In cotton host plant resistance is the best long term and explored strategy to protect the plants from CLCuD (Solomon-Blackburn and Bradshaw, 2007). Cotton leaf curl disease spread from the primary inoculum that is present in off season in the form of weeds and other hosts (Monga *et al.*, 2001). The management of CLCuD includes control of vector whitefly and eradication of weeds that contribute the hospitality of Cotton leaf curl virus (Monga *et al.*, 2001).

The seed treatment with systemic insecticides may prevent the cotton crop up to 50-60 days. By using insecticides even if infection occurs at later stage the severity of losses may be avoided as symptoms appearance will begin after 65-90 days and plants avoid the most susceptible stage (Monga *et al.*, 2011).

Growth and production of cotton is reduced by both abiotic and biotic stresses Zafar and Athar 2013. Biotic stresses or diseases affect uptake and utilization of nutrients by plants. It is therefore, plant mineral nutrition management not only used for producing higher crop productivity; it also used to change plant responses to biotic stresses disease incidences (Walters & Bingham, 2007). Various agronomic practices like sowing time and application of nutrients (Nitrogen and Potassium) can serve the purpose. The knowledge about K nutrition on association between plants and pests may help in developing strategies to set up high yielding production system by reducing disease incidence Zafar and Athar. 2013. Choosing best sowing time for a particular variety in different regions is difficult as too early and too late sowing may result in problems of diseases and pests. Appropriate sowing time preferably mid April to mid May results in decrease of disease incidence (Ghazanfar *et al.*, 2007) as compared to delay in sowing from mid May to June. Iqbal and Khan 2010 reported that increased plant spacing in case of early sowing and decreased plant spacing under late sown conditions is effective in management of CLCuD. They also concluded that CLCuD infestation reached its maximum after 105 days of sowing and in case of late sown crop i.e. 15 June or later infestation become severe after 45 days of sowing. They recommended 15 cm plant spacing in order to manage CLCuD in case of planting later than 15th of June.

According to Zafar *et al.* 2010 by understanding the physiological basis of nutrition (nitrogen) strategies can be designed to prevent, escape, avoid and control viral diseases. In case of resistant cultivars nitrogen concentration does not affect but in susceptible cultivars its concentration plays an important role to tackle disease severity. The most recommended management practices to tackle CLCuD disease include virus resistant cultivars, management of causal agents and mineral nutrition (Akhtar *et al.*, 2004). The influence of Potassium (K) application on disease through specific metabolic functions alters the relationship of host-parasite environment (Kafkafi *et al.*, 2001). Pervez *et al.* 2007 conducted an experiment on role of Potassium (K) in the control of CLCuD. According to their studies by increased application of Potassium upto 250kg/ha results in the reduction of disease from 12 to 38%. This increased application contributed considerably as seed cotton yield increased up to 37% as compared to Zero-K. ACoording to Farooq *et al.* 2013 the traits like plant height, bolls per plant and sympodia per plant may be considered for selection in virus intensive conditions as they found higher estimates of broad sense heritability along with positive and significant genotypic correlation with seed cotton yield in virus intensive environment.

Recent advances to combat clcuv through biotechnological tools

In plants lacking natural disease resistance PDR approach has been documented to combat different viruses. According to Hashmi *et al.* (2011) by exploiting transcriptional control two truncated forms of replicase (tACI) gene, capable of expressing only N-terminal 669bp (5'ACI) and C-Terminal 783bp(3'ACI) nucleotides were introduced into *Gossypium hirsutum* through cloning. A strain LBA 4404 of *Agrobacterium tumefaciens* was used through interference technology to impair cotton leaf curl virus in transgenic cotton. When transformed plants were compared with control non-transformed plants the over expression of either of the above mentioned nucleotides confer resistance by inhibition of viral genomic and β satellites DNA components. In early and late growth stages Northern blot hybridization revealed high transgene expression (Hashmi *et al.*, 2011).

Conclusion

Various aforementioned procedures of controlling CLCuD can be implemented depending upon situation. Development of resistant varieties along with Agronomic, fertilizer, insecticidal control and biotechnological methods can be used alone and in combination to control this severe disease which is still a challenge even after twenty years of extensive research.

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