

Review article

An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity

Amjad Farooq¹, Jehanzeb Farooq¹, Abid Mahmood¹, Amir Shakeel², Abdul Rehman³, Asia Batool⁴, Muhammad Riaz¹, Muhammad Tasdiq Hussain Shahid¹ and Saira Mehboob⁴

¹Cotton Research Institute, Ayub Agricultural Research Institute, Jhang Road, Faisalabad, Pakistan

²Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan

³Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

⁴Plant Pathology Section, Plant Protection Research Institute, AARI, Jhang Road, Faisalabad

*Corresponding authors: amjad1545@hotmail.com, jehanzeb1763@hotmail.com

Abstract

Cotton Leaf Curl Disease is among the most devastating natural calamity that inflicted huge losses to cotton crop productivity especially in Pakistan during the last 20 years. The dilemma of CLCuD is still under discussion among the researchers since its appearance in 1967. In 1992-93, CLCuD appeared in epidemic form which caused a decline in yield down to 9.05 million bales and during 1993-94, to 8.04 million bales in Pakistan. For screening against CLCuV to develop virus resistant cultivars disease was induced through grafting, delayed sowing and whitefly mediated transfer. The disease epidemiology is changed by abiotic factors especially temperature and plant age. Cotton leaf curl virus disease not only affect yield but also deteriorate fiber quality traits like Ginning out turn percentage, staple length, fiber uniformity index, fiber fineness, fiber bundle strength, maturity ratio because of change in composition of major fiber components including cellulose, protein, wax and pectin. The uncertainty of inheritance of CLCuD also prevails whether it is under the control of dominant or recessive genes which may be monogenic or polygenic whereas, extrachromosomal inheritance is also under discussion. The resistance breakdown depends upon the evolutionary potential of the pathogen and the 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. 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 aid 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: Cotton, Biotic stresses, whitefly, CLCuD, Geminiviruses, Begomoviruses.

Abbreviations: CLCuD= Cotton leaf curl virus disease, ssDNA= Single-stranded deoxyribonucleic acid, CLCuMV= Cotton leaf curl Multan virus, CLCuRV= Cotton leaf curl Rajasthan virus, CLCuAV= Cotton leaf curl Alabad virus, CLCuKV= Cotton leaf curl Kohran virus, PaLCuV= Papaya leaf curl virus (), CLCuBV= Cotton leaf curl Bangalore virus, CLCuBuV= Cotton leaf curl Burewala virus, CLCuShV=Cotton Leaf Curl Shahdadpur Virus, CLCuMV=Cotton leaf curl Multan Virus, CLCrV= Cotton leaf crumple virus, GOT%= Ginning out turn percentage, ELISA=Enzyme linked immunosorbant assay, PDR= Pathogen disease resistance, SI=Severity index, PDI= Percent disease incidence, RFLP= Restriction fragment length polymorphism.

Introduction

The environmental calamities including biotic and abiotic stresses are the major threats to agriculture and food security. Biotic stresses including viruses cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world as well as in Pakistan. A class of *Geminiviruses* was observed in 1978, on plants with distinct characteristics of size and appearance of geminate particles and was subsequently proven to be evidence that these are single-stranded deoxyribonucleic acid (ssDNA) virus (Mathews, 1987). The family *Geminiviridae* comprises of three genera i.e. *Mastrevirus*, *Curtovirus* and *Begomovirus*. A notorious group of these viruses belongs to genus *Begomovirus*, cause of 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 a persistent manner (Brown et al., 1995; Rybicki and Fauquet, 1998). Most of the *Begomoviruses* comprised of two genomic components called DNA-A and DNA-B, which are indispensable for a disease that is transmitted by whitefly *Bemisia tabaci* (Monga et al., 2011). There are numerous viruses from the Old World which have only a single constituent, analogous to DNA-A, which has been isolated and shown to bring on disease symptoms (Navot et al., 1991; Dry et al., 1993; Tan et al., 1995). The DNA isolated from an infected plant of cotton with CLCuD showed wide-ranging homology with the DNA-A component and other *Begomoviruses* in the Indian sub-continent (Zhou et al., 1998). Seven diverse species of

Begomoviruses have been found associated with the disease in the Indian subcontinent specifically Cotton leaf curl Multan virus (CLCuMV), Cotton leaf curl Rajasthan virus (CLCuRV), Cotton leaf curl Alabad virus (CLCuAV), Cotton leaf curl Kokhran virus (CLCuKV) and Papaya leaf curl virus (PaLCuV), Cotton leaf curl Bangalore virus (CLCuBV) associated with alpha-satellite and beta-satellite molecules (Mansoor et al., 2003b; Briddon et al., 2003) and Cotton leaf curl Burewala virus (CLCuBuV) (Amrao et al., 2010). CLCuD is recorded as one of the disparaging diseases of cotton. Cotton leaf curl virus has an attention-grabbing evolutionary story. It was first reported 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 (Farquharson, 1912; Hussain and Ali, 1975). It was not well thought-out as a serious disease up to 1987 but appeared in epidemic form in 1992-93 which caused a decrease in production down to 9.05 million bales and during the 1993-94 season to 8.04 million bales (Mahmood et al., 2003). The financial losses with the estimated value of \$5 billion (US) to the nation occurred from 1992-97 (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 incidence of CLCuD varies from year to year and also varies from area to area under cotton cultivation. Cotton belongs to genus *Gossypium*, which comprises of 52 species, of which four are cultivated species including *G. hirsutum* (Allotetraploid), *G. barbadense* (Allotetraploid), *G. arboreum* (Diploid) and *G. herbaceum* (Diploid) (Azhar et al., 2010b). The *hirsutum* species of cotton (Upland or American) are under the attack of Cotton leaf curl disease (CLCuD) since 1970 and ruined the existing variety S-12 and are still slaving the new emerging varieties (Perveen et al., 2010).

Symptoms

Cotton Leaf Curl Disease (CLCuD) infected plants may show a range of symptoms depending on the severity of disease, typical symptoms include thickening and yellowing of small veins on the lower surface of young leaves. Under severe attack of the disease, leaves curl downward or upward with stunted plant growth due to reduction of inter-nodal distance (Briddon et al., 2001; Qazi et al., 2007). The severity of disease also produces a cup shape outgrowth on the lower side of the curled leaves that is known as Enation (Mansoor et al., 1993; Harrison et al., 1997). The appearance of the disease at seedling stage seriously retards the flowering, boll formation, maturation, seed cotton yield and fiber quality (Rehman et al., 2000; Monga et al., 2011). CLCuD showing upward curling along with thickening of the leaves of the cotton plant (Fig.1), severe curling along with thickening of leaves (Fig.2), enations on the underside of the leaves (Fig.3) and stunting of the cotton plant (Fig.4).

Host range

Host range that has been identified for CLCuV include *Abutilon theophrasti* (Nill), *Althaea rosea* (Cav.), *A. ficifolia*, *A. kurdica*, *A. nudiflora*, *A. Pontica*, *A. sulphurea*, *G. barbadense*, *G. hirsutum*, *Hibiscus cannabinus* (L.), *H. esculentus* (L.), *H. ficulneus*, *H. huegelii*, *H. trionum*, *H. sabdariffa* (L.), *Lavatera cretica*, *Malva alcea* (L.), *M. silvestris* (L.), *M. moschata* (L.), *Malvaviscus arboreus* Car., *Pavonia hastate* (L.), *Sida acuta* (Burm.), *S. alba* (L.), *S.*

cordifolia (L.) and *Nicotiana tabacum* L. (Tarr, 1951, 1957; Bink, 1975; Cauquil and Follin, 1983; Fauquet and Thouvenel, 1987). *G. arboreum* and *G. herbaceum* are resistant to CLCuV (Cauquil and Follin, 1983). Similar to Cotton leaf curl virus symptoms were reported in other plant species in Africa but there is ambiguity whether the same virus is involved in these species or not. These include *Corchorus fascicularis* Lau. (Tilliaaceae), *Phyllanthus niruri* L. (Euphorbiaceae), *Clitoria ternatea* L. (Fabaceae), *Phaseolus vulgaris* (Fabaceae), *Sida urens* (Malvaceae), *Petunia* sp. (Solanaceae) and *Urena lobata* (Tarr, 1951; Nour and Nour, 1964; El- Nur and Abu Salih, 1970). In Pakistan under field conditions CLCuV symptoms were observed on alternate hosts like Brinjal, Cucurbits ("*Tinda*", "*Kali tori*"), *Convolvulus arvensis*, *Rumex dentatus*, Water Melon, Cow Pea and Lilly plants (Anonymous, 1993).

Epidemiology

Epidemiology of Cotton leaf curl virus disease (CLCuD) is affected by climatic conditions like rainfall, wind and temperature in Africa. Rainfall prior to seedling may result in the development of an increased population of vector due to abundance in food source (Bink, 1975). 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 a 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 a positive and significant correlation between % disease incidence and plant age. Maximum disease incidence % was recorded at 6 week old seedlings and it gradually decreased with increase in age of the plant. Many researchers found non-significant relationship of white fly population with disease (Briddon et al., 1998; Hameed et al., 1994; Iqbal, 1993).

Effect of CLCuD on yield components

Losses due to CLCuD are dependent on infectivity time and variety. The pronounced damage of CLCuD is in early stages but at later stages results minor infections (Brown and Bird, 1992; Akhtar et al., 2003b). 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 the last 20 years are given in Fig-5 and detailed area production and lint yield trends from 1947 to 2009 are given in Fig-6.



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



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



Fig 3. Enations on the underside of Cotton leaf.

Impact of CLCuD on fiber traits

The cotton fiber (lint) is the most important commodity in textile industry and CLCuD also affects fiber quality traits (Ali et al., 1995; Khan et al., 2000; Khan et al., 2001; Kalhoro et al., 2002; Khan et al., 2003). According to Ahmed (1999) CLCuD can decrease fiber length 3.44%, fiber strength 10% and elongation percentage upto 10%. Akhtar et al. 2009 studied the impact of CLCuD on fiber quality traits and their 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 effects of this viral disease on cellulose, protein, wax and pectin which are the major constituent of the fiber. But in view of Idris (1990) virus has significant impact on yield but not on fiber quality.

Inheritance of Cotton Leaf Curl Disease (CLCuD)

The inheritance of cotton leaf curl disease is still a dilemma among the plant researchers and no comprehensive assessment found about the resistance inheritance of this disease (Khan et al., 2007). The viral resistance in cotton may be an unstable character reported by Tarr (1951). The breeding for cotton leaf curl disease (CLCuD) resistance has been achieved through the assemblage of minor genes by recurrent selection (Hutchinson and Knight, 1950) and according to Azhar et al. (2010a) resistance depends on major genes (dominant genes) which may lose quickly because of the evolution of pathogen for these genes. An alternative approach is needed for partial resistance that depends on the recombination of minor genes (recessive genes). 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. (2006). The cross between *Gossypium barbadense* L. (Giza-45) and *Gossypium hirsutum* L. (Reba P-288) determined the effects of a single dominant gene supported by Aslam et al. (2000). The F_1 of crosses between highly susceptible S-12, highly resistant LRA-5166 varieties were found all virus free plants and their F_2 was close to 1:3 ratios which exhibit the presence of a single gene for the inheritance of resistance against CLCuD reported by Mahmood (2004) and Rehman et al. (2005). Whereas in the same cross (LRA-5166 \times S-12) no single gene of major effect found to be responsible for cotton leaf curl disease (Khan et al., 2007). Whereas the nuclear inheritance is under discussion, the extra chromosomal inheritance is also a secret and considered to be absent by Rehman et al. (2005) but the presence of reciprocal differences in the cross LRA-5166 is advocated by Khan et al. (2007)

Resistance Breakdown against CLCuD

The genetic resistance in plants is ruined due to the presence of resistance-breaking pathogen genotypes increasing frequently and this phenomenon depends upon the evolutionary potential of the pathogen. The three evolutionary forces i.e. population size, gene or genome flow (i.e. Migration) and reproduction or mating system (i.e. sexual or asexual) is further divided into three categories (1, 2 and 3) of predicted risk of each force and CLCuD comes under category 3 because of its maximum severity reported by García and McDonald (2003). The possibility of



Fig4. Stunting of the cotton plant.

recombination among the *Geminiviruses* and conducive environmental conditions increases the chances of new more virulent and resistance breaking variants of viruses knocked down the resistance (Shah et al., 1999; Chakrabarty et al., 2010). Those cultivars showed complete resistance against Cotton leaf curl Multan virus (CLCuMV) become susceptible to Cotton leaf curl Burewala virus (CLCuBuV) due to the emergence of more virulent new race of virus in this vicinity (Mahmood et al., 2003). As the phenomenon of recombination is responsible for the evolution of CLCuMV, CLCuBuV and similarly Cotton leaf curl Shahdadpur virus CLCuShV is a new recombinant sequence derived from *Begomovirus* species that were considered as epidemic of CLCuD in the Punjab during 1990, s (Amrao et al. 2010; Monga et al., 2011). Resistance durability is dependant if no resistance breaking has taken place or it is effective for 25 or more years (García and McDonald, 2003). In case of CLCuD resistance durability is limited and has not been taking place for more than 3 or 4 years as the parents CP-15/2 and LRA-5166 used in developing resistant cultivars in Pakistan have a narrow genetic base coupled with the arrival of new strains of the virus (Rahman et al. 2005; Padidam et al. 1999).

Resistance Source

The wild species of *Gossypium* are potential sources 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* has 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., 2010b).

CLCuD transmission and screening methods

CLCuD is transmitted by the *B. tabaci* and is associated with members of the *Geminiviridae*, genus *Begomovirus* and is not passed by transovarial means (Kirkpatrick, 1930, 1931; Nour and Nour 1964; El-Nur and Abu Salih, 1970; Mohsin et al., 1992; Markham, 1992). In some studies for transmission 3.5h acquisition-access feed and 30-min inoculation feed were required however, transmission threshold is reported as 6.5h (Kirkpatrick 1930, 1931). CLCuD symptoms can develop on inoculative plants in 15-30 days (Lagiere and Quattara,

1969). Cotton leaf curl virus disease is not transmitted by jassids, aphids, flea beetles or thrips (Kirkpatrick, 1931; Watkins, 1981). Commonly used screening methods include, the exploitation of virus spreader line (S-12) and white fly as a source of transmission vector (Shah et al., 2004; Perveen et al., 2005). For transmission through spreader line these researchers used S-12 the popular and most susceptible variety to CLCuD disease. This variety was planted in rows among the tested genotypes for natural spread 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., 2006; Perveen et al., 2010). They established CLCuD nursery near the experimental area to allow the spread of the 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 (Fauquet and Thouvenel, 1987; Akhtar et al., 2000, 2001, 2002c). Grafting as a successful method to inoculate CLCuD was used by Akhtar et al. 2004, 2010, Shah et al. 2004 and Mansoor et al. 2003. For grafting researchers employed three procedures like bottle graft, top cleft and wedge graft. In this procedure the stock used as resistance and the scion as a susceptible source for inoculation of disease and later presence of the virus was confirmed by the ELISA test. PCR can be used as a reliable tool for the identification of viruses. As the *Geminiviruses* are small, single stranded and have a circular genome thus PCR can be efficiently used for their detection. Several degenerate primers have been designed for the detection of these viruses (Rojas et al., 1993; Briddon and Markham 1994; Wyatt and Brown, 1996). 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 (Rybicki and Hughes., 1990, 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 the release of counted veruliferous flies on test plants under plastic jars in polyhouse for fixed interval (Monga et al., 2011). The pollen irradiation technique may be used as a criterion to develop CLCuV tolerance in cotton germplasm by creating genetic variability. Aslam and Elhai 2000 used pollen irradiation method and applied doses of 5-10Gy of gamma rays to create variation in the germplasm for resistance against Cotton leaf curl virus and also attempted different crosses of the irradiated germplasm. Similar method for creating variation was adopted by (Doak, 1934; Aslam et al., 1994; Aslam and Stelly, 1994).

Disease Ratings Scales and various Formulas to calculate severity and percent disease index for CLCuD in Cotton

The major problem for developing resistance against CLCuD is the lack of a standard method for the assessment of resistance. Viral diseases show different levels of infestation at different plant growth stages thus based on symptomatology and particular stage of plant growth different scales are classified regarding the severity of disease. The resistance level is usually described on severity basis of disease symptom which may be at any plant stage, inoculation pressure and growth conditions (Lapidot et al., 2006). The rating scales used numerically in different crops (tomatoes, chillies, tobacco etc.) likewise in cotton with slight

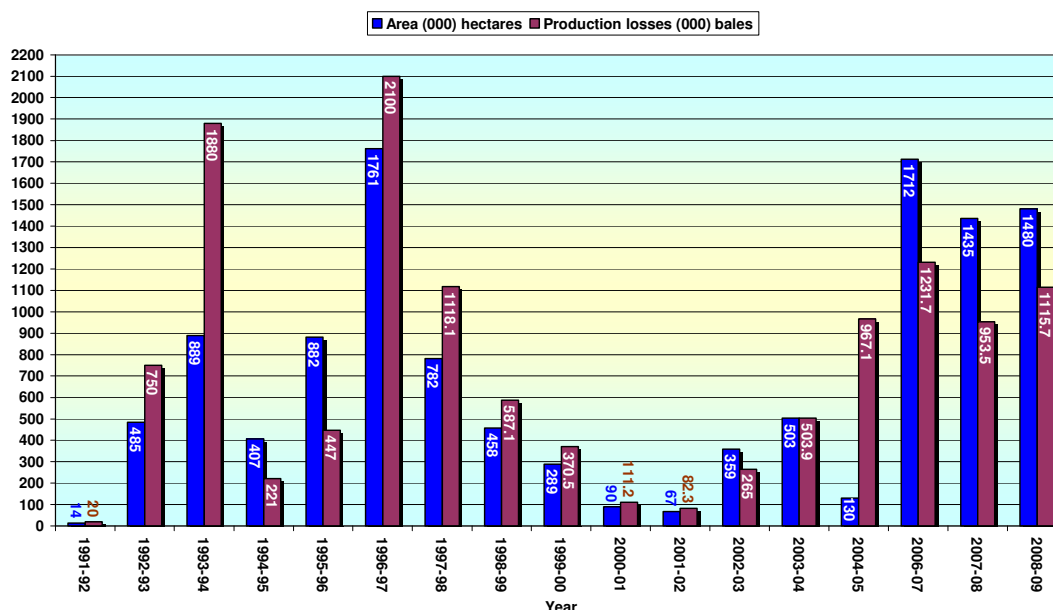


Fig 5. Affected area and production of Cotton due to CLCuV from 1991-92 to 2008-09 excluding 2005-06 in Pakistan

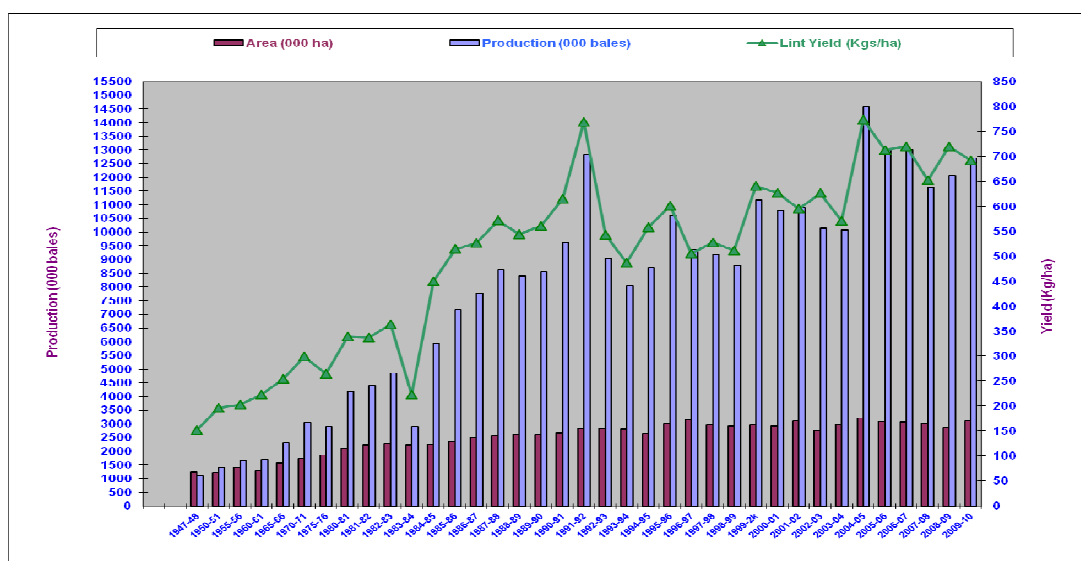


Fig 6. Area Production and Lint Yield of Cotton from 1947-48 to 2009-10 in Pakistan

modifications. The disease scale that has been generally used in cotton is based on Severity Index (SI) and Percent Disease Incidence (PDI %). The formula used to calculate both of these two parameters has been reported by Akhtar et al. 2003b, 2005, 2010. Akhtar et al. 2010 also used yield data to correlate it with SI and PDI %. According to this formula Individual symptomatic plant ratings for each genotype are summed up and divided by the number of infected plants to calculate the corresponding SI. This method of calculating disease incidence has been used in the tea plant for blister blight, in chilies for fruit rot, powdery mildew and in citrus

for citrus canker (Saravanakumar et al., 2007; Anand et al., 2010; Sahi et al., 2007)

$$\% \text{Disease incidence} = \frac{\text{Sum of all disease ratings of the selected plants}}{\text{Total no. of plants assessed}} \times 100/6$$

Another formula based on the number of infected plants but without any rating scale has been locally used to calculate percent disease incidence. This formula was used by Naveed et al. 2007. Formula is as follows.

$$\% \text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total no. of plants}}$$

The problem with this calculation method is that we cannot calculate the intensity of the disease and some of the genotypes may be ranked as susceptible even if they are showing less intensity. The formula given by Naveed et al. 2007 could not be applied efficiently for the calculation of disease as it is without any rating scale. The rating scale and formula given by Akhtar et al. 2010 is quite appropriate for the estimation of percentage and severity of Cotton leaf curl virus disease. Idris and Brown (2004) inoculated the cotton plants for the incidence of (CLCuV) through biolistic bombardment method on seedlings. By using this method seedling showed higher rate of infectivity i.e. 100%. This method can be applied in dissemination of CLCuD at seedling stage to calculate the incidence percentage of this viral disease.

Control measures (Non-Biotechnological tool) 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 (Jones, 2001; Solomon-Blackburn and Bradshaw, 2007). Cotton leaf curl disease spread from the primary inoculum that is present in the 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 (Narula et al., 1999; Monga et al., 2001). The seed treatment with systemic insecticides may prevent the cotton crop upto 50-60 days. By using insecticides even if infection occurs at a later stage the severity of losses may be avoided as symptom appearance will begin after 65-90 days and plants avoid the most susceptible stage (Singh et al., 2002; Monga et al., 2011). Various agronomic practices like sowing time and application of nutrients (Nitrogen and Potassium) can serve the purpose. Choosing a 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 the 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 becomes severe after 45 days of sowing. They recommended 15 cm plant spacing in order to manage CLCuD in the 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 causative 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 up to 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.

Recent advances to combat CLCuV through biotechnological tools

The non-biotechnological controlling methods have some pitfalls that fluctuate from climate to climate and also based on resources. With the introduction of plant biotechnology and genetic engineering, now it is easy to clone and evaluates different components of certain viruses and construct controlling strategies for reducing yield losses of corresponding crop plants. Diseases have caused considerable loss to yield during the domestication of plants from wild to cultivated forms (Agrios, 1997). For the development of resistance in plants, the main dilemma the plant breeder has to face is the introgression of resistance traits through conventional procedures of breeding. The crop plants today may have resistance developed by genetic engineering and this resistance is controlled by single or multiple genes against certain pathogens (Crute and Pink, 1996). In plants lacking natural disease resistance Pathogen disease resistance (PDR) approach by RNA mediated technology (sense and anti-sense RNA mediated) and protein mediated resistance has been documented to combat different viruses. Many genes have been incorporated in a number of plants to engineer PDR, especially in those crops where natural resistance genes are not found (Gallitelli and Accotto, 2001). 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 confers 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). The other approach to develop resistance is the presence of resistance genes in related plant species for certain viruses. These resistance inducing genes are generally present in different genotypes but need to be incorporated into the commercial varieties for efficient disease management (Kumaran, 2005). The wild *Gossypium* species namely *G. thurberii*, *G. anomalum*, *G. raimondii*, *G. armourianum*, and *G. tomentosum* are a good source of resistance to insect pests, such as boll- worms, jassids, whitefly and mites, and for resistance to diseases including bacterial blight, and Verticillium wilt (Azhar et al., 2010b). According to Briddon and Markham (2000) *G. arboreum* is free from CLCuD and to other fungal and bacterial diseases (Niu et al., 2008; Maqbool et al., 2008). Although genetic variation in *G. arboreum* is scanty (Rahman et al., 2007; Kantartzi et al., 2008) yet, it has the ability to resist against sucking pests such as white flies, thrips, leafhoppers and aphids. For the development of tolerance/resistance to various biotic and abiotic stresses, *G. arboreum* has been exploited in the isolation and incorporation of resistance genes into susceptible varieties through genetic transformation. The use of molecular markers associated with leaf curl virus (CLCuV) disease resistance in cotton has the potential to improve the efficiency of selection in cotton breeding programs. The advantage of DNA marker-based assay is that

the selection for resistance would be made without having to infect plants with the pathogen, thereby minimizing the possibility that the pathogen might escape into a new environment (Aslam et al., 2000). Aslam et al. 2000 evaluated a subset of F₂ plants by selective genotyping, with restriction fragment length polymorphism (RFLP) to identify DNA markers linked to the CLCuV resistance gene and found three DNA marker loci, linked to each other, also showed significant association with CLCuV resistance. According to them sequencing of linked markers will allow locus-specific DNA primers for exploitation in PCR-based detection of CLCuV-resistant plants in breeding populations.

Conclusion

All the above mentioned measures of controlling CLCuV can be implemented depending upon the conditions. 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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