Studies on Epidemiology and Molecular Diversity of Cotton Leaf Curl Virus (CLCuV) in Cotton (*Gossypium hirsutum* L.)

By

Prashant Kumar Chohan

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CERTIFICATE – I

This is to certify that this thesis entitled, "**Studies on epidemiology and molecular diversity of cotton leaf curl virus (CLCuV) in cotton (*Gossypium hirsutum* L.)**", submitted for the degree of **Doctor of Philosophy** in the subject of **Plant Pathology** to the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Prashant Kumar Chohan** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. Naresh Mehta)

Major Advisor

Professor
Department of Plant Pathology
CCS HAU, Hisar - 125004

CERTIFICATE – II

This is to certify that this thesis entitled, **Studies on epidemiology and molecular diversity of cotton leaf curl virus (CLCuV) in cotton (*Gossypium hirsutum* L.)**, submitted by **Prashant Kumar Chohan (Adm. No. 2012A44D)** to the Chaudhary Charan Singh Haryana Agricultural University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the subject of Plant Pathology, has been approved by the Student's Advisory Committee after an oral examination on the same.

MAJOR ADVISOR **EXTERNAL EXAMINAR**

HEAD OF THE DEPARTMENT

DEAN, POSTGRADUATE STUDIES

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CHAPTER- I

INTRODUCTION

Cotton is counted as one of the most important cash crop belongs to genus *Gossypium* contains nearly 50 species of which 44 are diploid (2n=2x=26) and 6 are allotetraploid (2n=2x=52). Four domesticated species *viz. Gossypium arboreum, G. herbaceum, G. barbadense* and *G. hirsutum* are grown worldwide. Cotton is cultivated in Indian subcontinent as commercial cash crop occupying an area of 12.7 million hectare ranked first in the world, whereas, ranked second in production with 25.50 million bales (170kg/bale) with average yield of 523 kg/ha (Anonymous, 2015). Cotton is grown in India in three distinct agroecological zones *viz*., north zone (Punjab, Haryana, Rajasthan and Western Uttar Pradesh), central zone (Gujarat, Madhya Pradesh, Maharashtra and Orissa) and south zone (Karnataka, Andhra Pradesh and Tamil Nadu). The northern zone is totally irrigated, while the percentage of irrigated area in the central and southern zones is much lower, the lowest being in the central zone which has nearly 60% of cotton area. It is also grown in small area in the eastern region in Sundarbans of West Bengal and in north-eastern states (Anonymous, 2009).

In Haryana, it is grown in an area of 0.64 million hectare with a total production of 2.2 million bales. The average yield of cotton in Haryana was 665 kg/ha (Anonymous, 2015). There are number of biotic and abiotic limiting factors affecting the crop potential. The crop face losses by attack of various insect pest, diseases and other edaphic factors that significantly affects the yield and quality of crop. Among the various diseases, cotton leaf curl disease (CLCuD) caused by cotton leaf curl virus (CLCuV) has been found to be the most important in respect of economic losses to the crop (Monga, 2014). The maximum area of cotton in Haryana is under *G. hirsutum* which is highly susceptible to CLCuD. CLCuV infected plants exhibit a range of symptoms such as small vein thickening at the time of disease initiation on younger leaves, main vein thickening with the progress of disease on entire leaf, leathery leaves, leaf curling (upward or downward), formation of extra leaf like structure at abaxial side called “leaf enation”. Under severe cases, the internodal length of plant get reduced and infected plant remained stunted and shows bushy appearance which affect the quality and production (Qazi *et al.*, 2007). The disease was first reported in India on *G. barbadense* at Indian Agriculture Research Institute, New Delhi in 1989 (Monga, 2014). Later in 1993, this was reported in Sriganganagar district of Rajasthan (Ajmera, 1994). The disease progressed to the entire cotton growing areas of north India within a short span of time (Rishi and Chauhan, 1994). Presently, this disease is very common in north India and cause severe economic losses

(Monga *et al.*, 2011). Reduction in height, number of bolls per plant, average boll weight and seed cotton yield are the main features in the diseased plants (Beniwal *et al.*, 2006). Considerable cotton seed yield reduction in Haryana (39.9 to 81.4%) has been reported by Monga *et al.* (2011). Weather variables such as relative humidity (RH) and temperature have shown major role in white fly population development which relating to progression of CLCuD (Sharma *et al.*, 2006).

Cotton leaf curl virus is a gemini virus (sub group III) belongs to the family geminiviridae of genus Begomovirus and is transmitted by its exclusive vector whitefly (*Bemisia tabaci* Gem.) in circulative and persistent manner (Sharma and Rishi, 2003). The virus causes major threat to cotton crop and presently the disease is known as cotton leaf curl disease (earlier cotton leaf curl virus disease) in cotton plant (Rybicki and Fauquet, 1998). Virus possesses a small circular, single stranded DNA (DNA-A) genome. In addition, a satellite virus molecules DNA β is also involved in the infection (Briddon *et al.*, 2001). Rajagopalan *et al.* (2012) have discussed the variations in genomic sequences, the virulence and infectivity of cotton leaf curl Burewala virus (CLCuBuV). Partial sequences of 258 and full-length sequences of 22 virus genomes were determined and it was observed that CLCuBuV have become the dominant virus in northern parts of India. The reports of breakdown of resistance to CLCuV in cotton have been reported in popular cotton cultivars in northwestern cotton belt of India (Anonymous, 2013). Six new strains of CLCuV were isolated during 2010 from northwestern cotton area of the country. Previously sudden incidence of new strain "Burewala strain" was observed in neighboring country Pakistan, the notorious strain that knocked down resistances of popular varieties there (Mansoor *et al.*, 2003). Centre of diversity and pandemic of CLCuV strains routed to India as shown in Fig 1.1 suggested the infiltration and spread to neighboring countries (Regenmortel, 2009). Accumulation of recombination events over the years coupled with favorable environmental conditions appeared to have knocked down the resistance of cotton during 2009-10 season in India (Chakrabarty *et al.*, 2010). Previously resistant varieties of cotton exhibited susceptible reaction and new strain (Sriganganagar strain) was isolated from the infected cultivar and the sequence analysis of DNA-A & β-DNA components were determined. The comparison revealed 81-99% and 88.3-92% identity of DNA-A and β-DNA respectively with known CLCuV sequences of isolates from Indian and Pakistan (Chakrabarty *et al.*, 2011).

Role of the major climatic conditions like temperature, wind, rainfall, RH (%), light and additionally of sex of white flies and plant age on the incidence and severity of CLCuD has been reported previously (Iqbal *et al.*, 2014). Rainfall before the seedling stage of cotton plant significantly increases the population of vector whitefly (*Bemisia tabaci*) due to increase in food sources (Bink, 1975). The source of CLCuV infected plant imparts significant effect on secondary spread and for that the primary source of

infection is required for initial whitefly population. The primary sites of infection have been 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). Once the virus is established in a particular area through its vector, the secondary infection occurs by spread through huge population of whitefly in cotton field during whole growing season (Giha and Nour, 1969). The correlation studies on role of weather parameters on epidemiology of CLCuD were conducted regularly by various researchers. Non-significant co-relation has been observed at minimum air temperature, relative Humidity, wind velocity, sunshine hours, rainfall and whitefly population on different varieties for the development of cotton leaf curl disease (Akhtar *et al.*, 2002). Various independent studies indicated a non- significant relationship of whitefly population with the disease development (Briddon *et al.*, 1998; Hameed *et al.*, 1994; Iqbal, 1993).

The CLCuD of cotton crops was primarily managed by the use of pesticides against vector whitefly to reduce virus transmission. The lack of alternative control options has led to the more and more and often over use, of pesticides to reduce vector populations. Non judicious use of chemicals against insect pest led to set a mentality of farmers to depend on pesticides only. This overuse of pesticides lead to the development of insecticide resistance in the whitefly vector, which subsequently undermines abatement of virus transmission to reduce damage and losses. As a consequences, in the present scenario use of chemicals has not been found effective for longer period for the management of disease. Central Institute for Cotton Research, Hyderabad (India) released advisory for the management of major diseases and insect pests also. Use of pigeonpea, bajra, maize or sorghum was suggested by CICR to minimize the incidence of whitefly in cotton, therefore, lowering the incidence of CLCuD also (www.cicr.org.in). The management through agronomic practices can help to escape from the disease. Appropriate sowing time, preferably mid-April to mid-May resulted in reduced disease incidence (Ghazanfar *et al.*, 2007). Increased plant spacing in the case of early sowing and decreased plant spacing under late sown conditions has been found effective in management of CLCuD (Iqbal and Khan, 2010). However, spacing difference in plants did not impart major role in management of CLCuD always (Iqbal *et al.*, 2007). The practice of maintaining more plant population with closer plant spacing could impart satisfactory yield even under the CLCuD infested field (Iqbal *et al.*, 2012).

It is feasible that the whitefly vector infesting a leaf curl non-host plant could harbor the virus complex, if ingested and acquired from an infected plant prior to dispersal to a non-host. Various weeds have been identified that proved to harbor the viral genome inside them. The CLCuV DNA has been recovered and the infection was exhibited from various alternate and collateral host weeds (Maharishi,

2015). The exact mechanism of resistance against CLCuD is still the matter of study, however, some physiological and anatomical changes might occur during the infection with the virus (Ashfaq *et al.*, 2007). Therefore, some of secondary metabolites produced in plant also have been reported to show change in their concentration under the attack of CLCuV. Diseased leaves shows reduction in chlorophyll content as compared to disease free leaves which reported more content of chlorophyll-a and b (Asharf and Zafar, 1999 and Ajmal *et al.*, 2011). Similarly, other secondary metabolites such as phenols, tannin and carotenoid concentration also increased in resistant and disease free plants (Beniwal *et al.*, 2006 and Ajmal *et al.*, 2011). Whereas, changes in sugar content in CLCuV infected susceptible and resistant plant showed non significant effect on imparting any resistance (Ashfaq *et al.*, 2014). Though, diseased plants showed high oil content along with its hydrolyzing enzyme lipase, however, resistant cultivar showed high protein content (Ashraf *et al.*, 2004). The enzymes oxidase and polyphenol oxidase (PPO) have shown increased activity in resistant genotypes (Siddiqe *et al.*, 2014).

It has been reported that resistance to any virus depends on plant metabolism (Dawson and Hilf, 1992), therefore, development of resistant varieties is the cheapest source for the management of plant viruses with keeping this thought that changes in biochemical processes due to viral infections must be recognized. Currently, sources of genetic resistance are lacking to manage the recently emergent, recombinant CLCuBV that predominates in northwest India. Several factors including evolution of new variants of the viruses, appearance of efficient vectors, weather events, changing cropping systems, movement of infected planting material and introduction of susceptible plant varieties individually, or in combination, have contributed to the emergence of begomovirus problems around the world (Varma and Malathi, 2003).

Keeping in view the potential threat of CLCuV in cotton growing area in north India as well as scanty information available on diversity of CLCuV as well as epidemiological factors responsible for disease development, the present investigations were carried out at CCS Haryana Agricultural University, Hisar with the following objectives:

1. To study the role of epidemiological factors on the development of CLCuD
2. To evaluate the effect of agronomic practices on incidence of CLCuD
3. To evaluate the effect of CLCuV infection on biochemical constituents of cotton
4. To analyse molecular diversity among CLCuV isolates

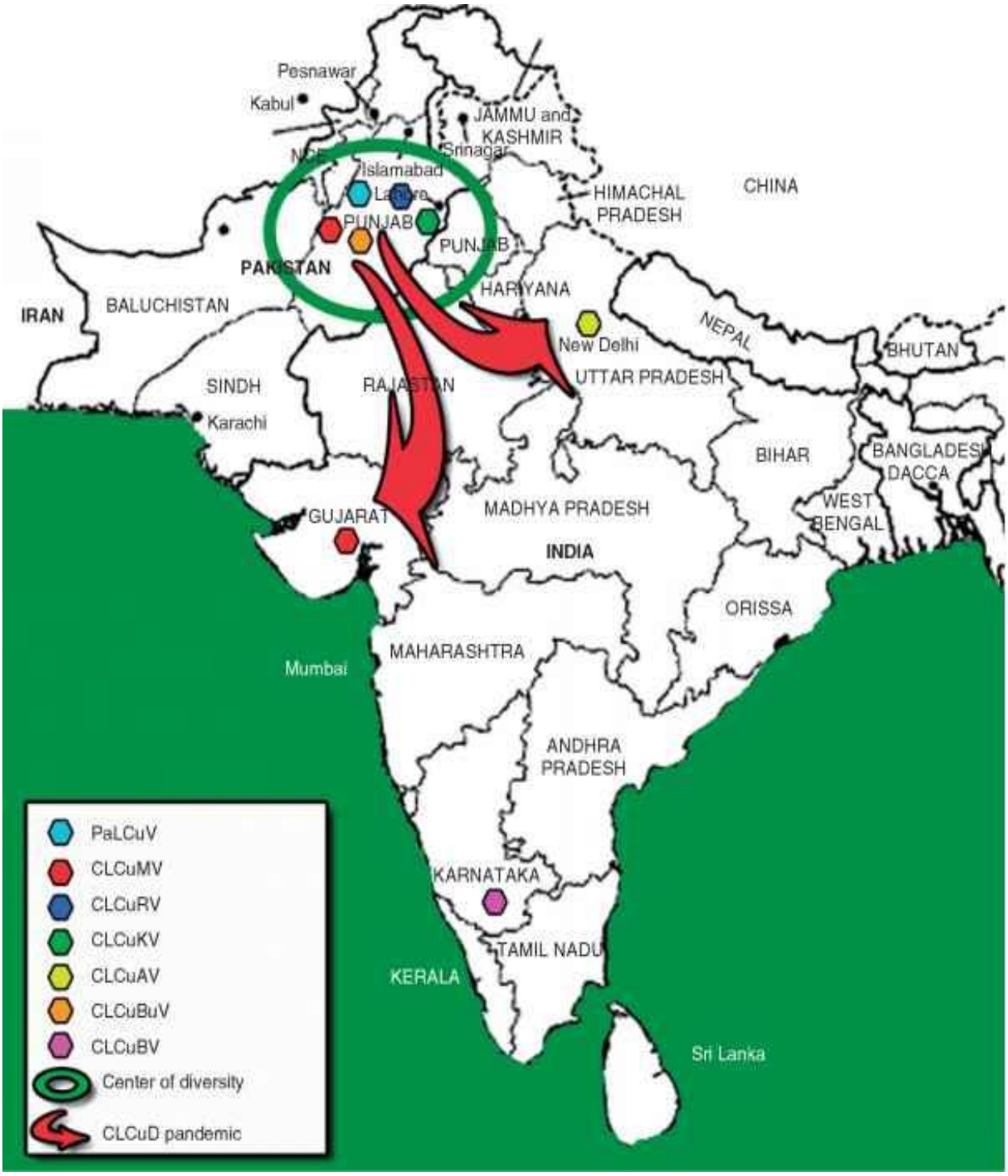

Fig 1.1 Localization of cotton leaf curl geminiviruses in the Indian subcontinent. PaLCuV, papaya leaf curl virus; CLCuMV, cotton leaf curl Multan virus; CLCuRV, cotton leaf curl Rajasthan virus; CLCuKV, cotton leaf curl Kokhran virus; CLCuAV, cotton leaf curl Allahabad virus; CLCuBuV, cotton leaf curl Burawalia virus; CLCuBV, cotton leaf curl Bangalore virus (Regenmortel, 2009).

CHAPTER-II

REVIEW OF LITERAUTURE

The disease (CLCuD) has created epiphytotic conditions many times in past and presently in the north western cotton growing areas of India. Studies on epidemiology, cultural practices in association of biochemical and molecular analysis may incite some consistent information on nature of CLCuV. Scanty information and limited literature is available on the related aspects of proposed investigation "Studies on epidemiology and molecular diversity of cotton leaf curl virus (CLCuV) in cotton (*Gossypium hirsutum* L.)", however, the literature relating to the present study under the following heads are being summarized as follows:

2.1 Importance

Cotton leaf curl disease (CLCuD) has about a centuary of history of its occurrence. The disease was reported for the first time from Nigeria on *Gossypium peruvianim* and *G. vitifolia* in 1912 by Faquharson, who reported that cotton leaf curl is a viral disease and caused by *Gossypium virus-1* and named it as African leaf curl of cotton. Later in 1924, the disease was reported from Sudan and Tanzania (Jones and Mason 1926; Kirpatrick, 1930a and 1931), thereafter disease spread to all the African countries situated north of equator except Egypt, Maghreb, Benin, Chad, Togo and Barkina Faso (Tarr, 1951). The leaf curl and mosaic virus complex in the African cotton belt later severely started affecting cotton (*G. hirsutum*) and in the Indian subcontinent (Fig 2.1), first time its incidence was observed in few plants of a newly introduced variety of *G. hirsutum* (Tahir *et al.*, 2011) in Multan province of Pakistan in 1967 (Hussain and Ali, 1975). Initially it was sporadic and became prominent with the time and brought down the cotton production in Pakistan (Mansoor *et al.*, 2011). The exact mode of entry in to Indian subcontinent of this virus has not been mentioned in the literature, however, in recent, the route of CLCuV movement has been studied and plotted (Fig 2.1 and Fig 2.2) by researcher through phylogenetic studies (Tahir *et al.*, 2011 and Saleem *et al.*, 2016).

In India, the disease showed its presence in the year 1989 and appeared at two different locations Bangalore, Karnataka (Nateshan and Muniyappa, 1992) and IARI, New Delhi (Ajmera, 1994) on *G. barbadense*. Later on, disease appeared on *G. hirsutum* near Sriganganagar in Rajasthan in 1993 (Ajmera, 1994) and in most of cotton area of northern India. In Haryana disease was first reported by Rishi and Chauhan (1994). Presently, it became a major threat to cotton cultivation in northern India (Monga, 2014)).

Fig 2.1 The possible route of CLCuV from Africa to Indian subcontinent through Pakistan (Tahir *et al.*, 2011)

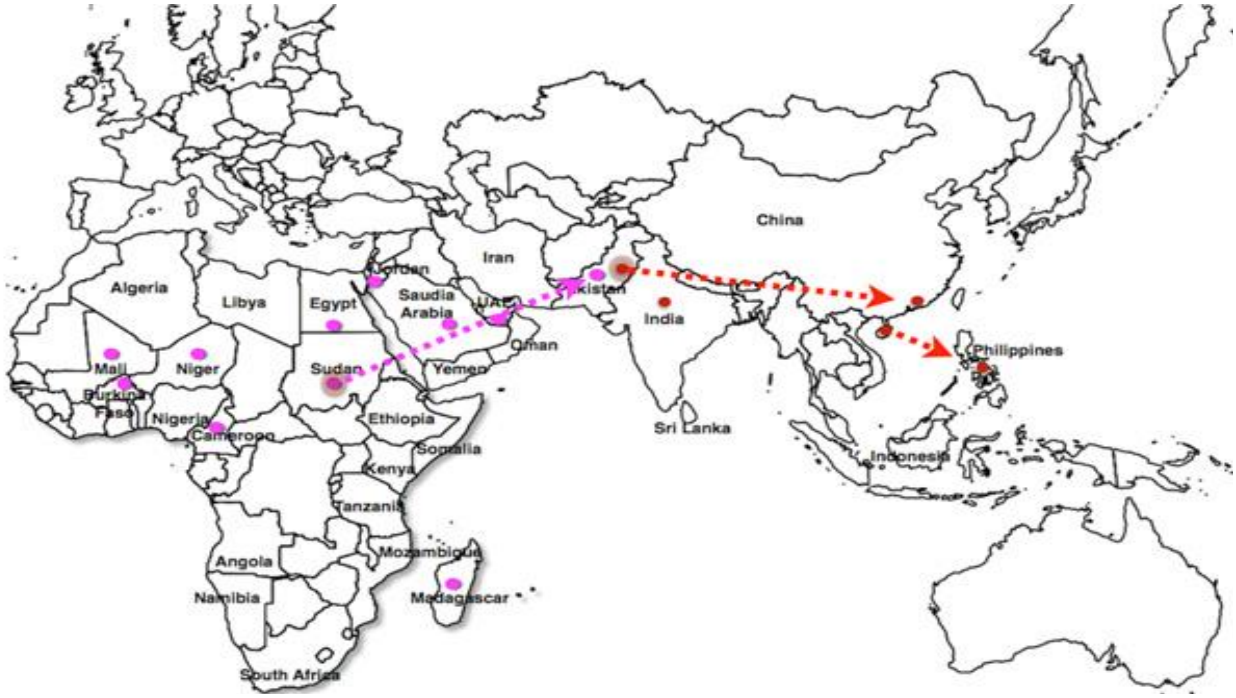

Fig 2.2 The centre of diversity of CLCuD in Asia was reported from Pakistan and India. Red marks indicating CLCuMuV movement and pink circles indicating CLCuGeV spread (Saleem *et al.*, 2016)

2.2 Symptoms of CLCuD

Symptoms of CLCuD may vary from variety to variety, however, a specific virus can produce similar symptoms in different hosts (Kirkpartrick, 1931). Different types of symptoms in *G. barbadense* involved spirally twisted petioles and fruiting branches, tall stems and elongated internodes (Tarr, 1951). Cotton leaf curl Gezira virus (CLCuGV) infected plants in Africa exhibited dwarfing, overall stunting, reduced boll number and boll weight (Farquharson, 1912 and Golding, 1930).

In *G. hirsutum*, infected plants showed two types of vein thickening (main and small veins) after infected with CLCuV. Main vein thickening was described by the dark green thickening of the distal end of the large veins of younger leaves while thickening of small veins was characterized by dark green or pale green thickening of fine veins of young leaves. These irregular thickenings of veins gradually extend and resulted in to reticulation of veins (Nour and Nour, 1964; Watkins, 1981).

As virus belongs to genus, Begomovirus that generally produce three types of prominent symptoms: leaf curl, yellow vein and yellow or golden mosaic. Enation formation, thickening of veins and upward or downward curling of leaves are typical symptoms of CLCuD (Mansoor *et al.*, 2003). However, typical symptoms of disease includes upward/downward leaf curling, dark green veins, vein thickening and enation (small fine leaf like structures) on the under surface of the leaves (Bink, 1975; Briddon and Markham, 2000). The severely affected plants showed bushy appearance with dark green canopy and short internodes without flowers and bolls (Chauhan, 2004).

2.3 Losses due to CLCuD

Cotton leaf curl disease being the most devastating natural calamity that reduced the quality and production of the cotton (Akhtar*et al.*, 2015). In India CLCuD caused yield loss to the tune of 79 per cent depending upon the stage of infection and cultivar (Hundal *et al.*, 2011). Disease has been reported to cause considerable seed cotton yield reduction in Rajasthan (32.9to 50.3%), Punjab (10.5 to 92.2%) and Haryana (39.4 to 81.4%) (Narula *et al.*, 1999; Monga *et al.*, 2001). Losses due to CLCuD on Bt cotton hybrids in north zone during 2009-12 ranged between 25.2-46.6 %.

Disease incidence remained low on early planted crop as compare to late planting. Maximum CLCuD incidence appeared within 30-45 days in late planting as compared to 105 days of early planted cotton. Delay in sowing, also reduced cotton seed yield progressively (Hussain *et al.*, 2015; Iqbal and Khan, 2010). CLCuD exhibited negative and significant association with seed cotton yield, ginning out turn (GOT) and number of bolls (Saeed *et al.*, 2014 and Farooq *et al.*, 2013). CLCuD severely hampered the crop performance in planting of cotton delayed from 15th April and increased the chances of disease incidence (Ali *et al.*, 2014). GOT, fiber length, fiber uniformity index, fiber fineness, fiber bundle

strength, maturity ratio also deteriorated because of change in composition of cellulose, protein, wax and pectin (Farooq *et al.*, 2014).

2.4 Geminiviruses

According to International Committee on Taxonomy of Virus (ICTV) "Viruses are elementary particles that possess some of the properties of living system such as having a genome and being able to adapt to changing environment". The study on virus showed about 650 known plant viruses (75% of the plant viruses) have been economically important pathogens, possess single stranded positive sense RNA genome, others have genome of negative stranded RNA, double stranded RNA, single stranded DNA or double stranded DNA (Garr *et al.*, 1992, Harrison and Murrnat, 1996).

In 1970s a distinct group of single-stranded DNA viruses was found associated with leaf curl symptoms (Harrison *et al.*, 1977) that was later named as geminivirus (Goodman, 1977). In 1980s the virus was classified into a new plant virus family Geminiviridae (Rybicki, 1994). Geminiviruses bisegmented (geminate) in shape and 30x20 nm in size. In 1978 ICTV recognized Geminiviruses as a separate group on the basis of geminate morphology of the virus particles and circular ssDNA as their genomic component (Rishi, 2004).

Viruses in the family Geminiviridae are characterized structurally by twinned (geminate) quasi-icosahedral capsids and genetically by having one or two small circular, ssDNA molecules. The family Geminiviridae consists of four genera of viruses namely, **Begomovirus**, Curtovirus, Topovirus and Mastervirus, based on genome organization, insect vector and host range (Padidam *et al.*, 1995). Geminiviruses replication occurred through an intermediate dsDNA molecule in the nuclei of infected host plant cells that completely depends upon the host DNA replication machinery (Jeske, 2007). Some geminiviruses were transmitted by whiteflies and others by leafhoppers in a persistent manner but not replicated in their vectors and also were not seed transmitted (Bock, 1982). They infected a broad range of plants including both monocots and dicots (Gutierrez, 2000). Most whitefly transmitted geminiviruses possess bipartite DNA genomes, DNA A and B.

2.4.1 Cotton Leaf Curl Virus

The virus which infected dicotyledonous hosts bean in central America and hence derived its name from type member **Be**an **go**lden **mo**saic i.e. “Begomo” virus. Based on genomic make-up, begomoviruses were sub-divided into two types, mono or bipartite genome (Seal *et al.*, 2006; Brown *et al.*, 2012). The bipartite genome consisted of two circular single stranded DNA of sized range 2.5 to 2.7 kb (DNA A and B). Both components have been independently encapsidated and together acquisition by white fly required for successful expression of virus in host. Bipartite viruses included Mungbean yellow

mosaic India virus (MYMIV). Monopartite genome consisted of only DNA A that showed infection and produced typical symptoms, for example, Tomato yellow leaf curl virus-Israel (TYLCV-Is), Tomato leaf curl Karnataka virus (ToLCKV) and Cotton leaf curl virus (CLCuV) have been the main viruses (Snehi *et al.*, 2015).

The features of transmission by white fly, association of geminate particle with diseased plant, hybridization of CLCuV A probe and PCR amplification with specific primers of DNA A of the virus (Mansoor *et al.*, 1998, Sharma and Rishi, 2007) showed that CLCuD is associated with a Begomovirus which presently very well-known with the name cotton leaf curl virus (CLCuV).

Though, approximately 288 species of Begomovirus have been identified. Among them, seven diverse species were found associated with the CLCuD in the Indian subcontinent specifically Cotton leaf curl Multan virus (CLCuMuV), Cotton leaf curl Rajasthan virus (CLCuRV), Cotton leaf curl Alahabad virus (CLCuAV), Cotton leaf curl Kokhran virus (CLCuKV) and Papaya leaf curl virus (PaLCuV), Cotton leaf curl Bangalore virus (CLCuBV) associated with alphasatellite and betasatellite molecules (Mansoor *et al.*, 2003b; Briddon 2003). The cotton leaf curl Burewala virus (CLCuBuV) which was identified in C49 isolate collected from CLCuD symptomatic cotton plant in Layyah district, Panjab (Pakistan) was appeared to be a new strain of Begomovirus (Amrao *et al.*, 2010). Two more viruses Tomato leaf curl Bangalore virus (ToLCBV) and Tomato leaf curl Palampur virus (ToLCPaV) were also identified as new cotton leaf curl begomoviruses of cotton (Verma and Malathi, 2003; Malathi, 2015). Later studies showed the recombination in several regions of DNA-A and β-DNA in Sri Ganganagar strain of CLCuV (Chakrabarty et al, 2011 and Malathi, 2015).

PCR-mediated amplification showed the presence of a begomovirus named it Cotton Leaf Curl Virus (CLCuV) in CLCuD-infected cotton. A smaller (approx. 1350 nucleotides) single standard DNA molecule (termed DNA-1) was shown to be associated with CLCuD (Mansoor *et al.*, 1999). Major DNA-A component encoded for viral functions required for replication and also played an essential role in insect transmission. Whereas, the second component DNA-B, encoded the products involved in movement within and between the cells in host tissues (Briddon, *et al.*, 2001 and Radhakrishnan *et al.*, 2004). Whereas, the new strain CLCuBV was lacked an intact transcriptional activator protein (Shuja *et al.*, 2014).

2.5 Whitefly in relation to CLCuD incidence

Like other Begomoviruses, Cotton leaf curl virus also vectored by whitefly (*Bemisia tabaci* Gen.) in circulative and persistent manner (Sharma and Rishi, 2003). Whitefly has wide range of hosts including vegetables, fibre crops, ornamental plants, pulses, horticultural plants and weeds (Oliveira *et al.*, 2001 and

Jones, 2003). The efficiency of whitefly to act as vector depends on two major phenomenon i.e. acquisition of virus from diseased plant and transmission of acquired virus into healthy host (Gupta *et al.*, 2010a). Incidence of CLCuD showed strong positive correlation with whitefly population (Sharma *et al.*, 2006). In addition to cotton (*G. hirsutum*), whitefly has also been a serious pest reported from horticultural and ornamental crops (Dittrich *et al.*, 1985). It caused extensive damage through direct feeding, honeydew production and as a viral vector. "Stickiness" in cotton, a major problem affecting throughout in cotton ginning and spinning mills was thought to be caused by the deposition of sugars by insects, particularly aphid and whitefly, on the open boll (Barton *et al.*, 2005). The disease has not been soil or seed borne therefore, viral infection depends primarily on the immigration of vectors from alternate hosts which acted as a reservoir of virus. The incidence and intensity of disease (CLCuD) was depend on cotton genotypes, ecological conditions and management practices (Iqbal and Khan, 2010). Weather parameters such as temperature, relative humidity considerably affected the shift in population of insect pests from small population to large population and ultimately affected the crop at any stage that resulted into significant yield losses (Hussain *et al.*, 2015). The viral capsid protein played important role in transmission by whitefly. The one of the part of coat protein encoded protein was responsible for the transmission of whitefly and "A" biotype of whitefly possess specific binding site for active LIVY virion (Whitfield *et al.*, 2015).

2.6 Agronomic practices in relation to CLCuD and effect on yield

Use of agronomic practices for the management of viral diseases has been discussed by various researchers. A disease map for CLCuD was suggested to be very helpful to start with the idea of study the variations in occurrence of this disease (Monga, 2014). Reduction in height, number of bolls per plant, average boll weight and of seed cotton yield were recorded in CLCuD infected plants in comparison to the healthy plants (Beniwal *et al.*, 2006). The sowing time difference i.e. normal and late sowing affected the incidence and disease index of CLCuD (Ahuja *et al.*, 2006; Perveen *et al.*, 2010). Appropriate sowing time, preferably mid-April to mid-May resulted in decrease of disease incidence (Ghazanfar *et al.*, 2007). Studies by Iqbal *et al.* (2007) suggested that severe infection of cotton plant with CLCuV can be managed by increasing plant population and nitrogen doses to achieve optimum seed cotton yield. Increased plant spacing in the case of early sowing and decreased plant spacing under late sown conditions has shown effective management of CLCuD (Iqbal and Khan, 2010). In CLCuD infected field, the cotton crop sown with 15 cm spaced plants resulted in maximum seed cotton yield only due to highest plant density (88,888 plants ha-1) as compared to cotton sown with 30 and 45 cm spaced plants (44,444 and 29630 plants ha-1,

respectively) which had more number of bolls per plant. Therefore, plant spacing has shown non-significant effects on boll weight and CLCuV infestations (Iqbal *et al.*, 2011).

Early sowing of cotton crop coincided with favourable environmental conditions for vigorous plant growth and hence resulted in to maximum plant height, number of nodes per plant, more number of bolls per plant, higher boll opening %age and finally more yield (Hussain *et al.*, 2015). The genotypes that were severely affected by CLCuD have been managed with increasing plant population (Iqbal *et al.*, 2005). Plant density also have shown role in incidence of viral diseases. In chickpea plants, low plant density (5 plants/m^2) exhibited the highest incidence of virus symptoms and the incidence declined as plant densities increased (Verrel and Moore, 2015).

2.7 Biochemical parameters

The host-pathogen interaction takes place with a struggle between both, however, in some cases interaction is seen very complex. Pathogen always try to stay alive within host and multiply with time, whereas, host plant tries to defend any damage occurring within it by producing some chemical barriers against the pathogen activities. Plants with a specific defense system against a particular pathogen shows absence or presence of some constituents that directly or indirectly are involved in resistance of host plant.

Whiteflies may differs in acquiring in virus could be due to some unknown biochemical functions related to the genetic differences amongst whitefly types or the host effect (Yang *et al.*, 2011). The difference in composition of sap from different host plants can influence the growth and development of sucking insects (Gupta *et al.*, 2010b), therefore, new cultivars with the aim of differences in biochemical compositions are to be produced for better management of CLCuD and its vector whitefly.

2.7.1 Phenols

Phenolic substances are known to influence various physiological processes such as oxidation reduction reactions, lignification and stimulation as well as inhibition of auxin activity. They may occur in simple and complex forms such as cinnamic, coumarine, caffeic, protocatechuic, chlorogenic and quinic acid that exhibit antimicrobial activities. Positive correlation between the amount of phenolic content and degree of resistance to plant disease has been evidenced by several workers.

Increase in 69 per cent of total phenol content was reported in resistant cultivars 101-102B of cotton against bacterial blight pathogen *Xanthomonas compestris* pv. *malvacearum*(xcm) as compared to the leaves of susceptible cotton cv. Acala-44 (Borkar and Verma, 1991). Multiple foliar disease resistant

sorghum genotypes also showed higher content of phenols compared to the susceptible genotypes (Kalappanavar and Hiremath, 2000).

2.7.2 Gossypol

It is present in gossypol glands of plant. High quantity of gossypol is undesirable for human/animal consumption however, this also confer resistance in cotton for insect pests including cotton bollworm, spotted bollworm (*Earias insulana*) and cotton leaf worm (*Spodoptera littura*). It also confer resistance against disease causing pathogens such as *Verticillium dahlia*, *Fusarium oxysporeum* and root knot nematode (*Meloidegyne incognita*). Gossypol imparts antibiosis type of resistance mechanism to the host plant.

In cotton, a high level of gossypol, flavanols, silica and low sugar contents were reported to have some role in insect resistance (Singh and Agarwal, 1988 and Hedin and McCarty, 1990). Gossypol glands constituted gossypol acted as insecticide, repellant and growth retardant in cotton (Wilson and Smith, 1976). Deleterious effect on bollworms/spotted bollworms have been reported (Duhoon *et al.*, 1981; Ilango and Uthamasamy, 1989). More susceptibility in glandless cotton than the glanded cotton cultivars were reported previoiusly (Jenkins *et al.*, 1966). The phenol, gossypol and tannin content decreased as the plant growth stage progressed from vegetative to reproductive phase (Acharya and Singh, 2008).

2.7.3 Sugar

Sugar plays important role in defense mechanism of plants as it is the precursors for synthesis of phenols, phytoalexins, lignin and callose in plant. Sugar content increased with the increased severity of late leaf spot of groundnut and the rate of increase was more in susceptible variety than in resistant variety (Bhat, 1997). Grey mildew infected cotton plants showed rapid decline of sugar content as compared to resistant and immune plants (Chakrabarty *et al.*, 2002). Higher sugar content was reported in CLCuD susceptible cultivar HS 6 than resistant cultivar H 1226. Total sugar was decreased in susceptible plants under increasing disease severity (Beniwal *et al.*, 2006). Non-Bt genotypes recorded lower amount of total sugar (-12.17 to -15.59%) and reducing sugar (-11.01 to -19.05%) compared to Bt genotypes. High amount of non-reducing sugar (13.18%) content at early stage (90 DAS) and lower amount (-27.04%) at later stage (120 DAS) was observed as compared to Bt genotypes (Ajmal *et al.*, 2011).

2.7.4 Protein

Plants with lower proportion of specific protein are less prone to disease even under favorable conditions. In the study on non-Bt genotypes Laxmi, Abhadita, DCH-32 and Bt genotypes RCH-2 Bt, JKCH-1 Bt and JKCH-2 Bt against Bacterial blight disease, non-Bt genotypes recorded high amount of

total protein (9.18 to 13.74%) as compared to Bt genotypes. Further, the decrease in total protein (-23.28 to - 25.17%) was more in infected plants of Bacterial blight disease as compared to the disease free plants (Govindappa *et al.,* 2008).

2.7.5 Chlorophyll

Studies on chlorophyll concentration during CLCuD attack on cotton showed the increase in chlorophyll-a content in the resistant cultivar as compared to the susceptible cultivars (Ajmal *et al.*, 2011; Zafar and Athar, 2013). Similarly, in biochemical analysis of secondary metabolites such as phenol, sugar, chlorophyll content etc. in cotton plants showed higher concentration of chlorophyll-a and chlorophyll-b in leaves of CLCuD resistant plants as compared to the susceptible plant (Anuradha, 2014). Whereas, in blackgram, Ashfaq *et al.* (2014) observed high chlorophyll content in susceptible cultivar as compared to the resistant cultivar against urdbean crinkle virus disease of blackgram.

2.7.6 Enzyme activity

Past studies showed that in addition to phenolic compounds, proteins and chlorophyll, enzyme activity also played important role in resistant against the plant pathogens. The activities of enzymes peroxidase and superoxide dismutase (SOD) was increased and decreased significantly after 15 and 30 days of urdbean leaf crinckle virus (ULCV)-inoculation in resistant genotype, respectively (Ashfaq *et al*., 2010). The Enzymes such as polyphenol oxidase (PPO), peroxidase and catalase have shown active role in disease resistance against *Ascochyta rabiei* (Kumar *et al*., 2013). In their studies calli of different chickpea cultivars were screened against *A. rabiei* and the calli of tolerant cultivars showed higher concentration of enzymes PPO and peroxidase. Siddique *et al.* (2014) also exhibited the role of various enzymes and their activity inciting resistance against the virus. Increase in total phenols, proteases, MDA, and PPO was reported in leaves of CLCuBuV-inoculated plants resistant genotypes and in non-inoculated plants. Whereas, their activity was decreased in susceptible genotypes. Increase in activity of protease, MDA, SOD, and PPO in resistant genotype *Ravi* after infection with CLCuBuV, suggested that there is a correlation between constitutive induced levels of these enzymes and plant resistance that could be considered as biochemical markers for studying plant-virus compatible and incompatible interactions (Siddique *et al.*, 2014).

2.8 Genome of CLCuV monopartite and new strains

A large number of monopartite bgeomoviruses have been found associated with ssDNA molecule. Half the size of genome (1.3kb), was required for typical symptom production in primary host known as beta (β) satellites. Genome of CLCuV was monopartite genome, but in some species of CLCuV β-satellites

were associated with some monopartite begomoviruses, including cotton leaf curl Multan virus (CLCuMV), tomato yellow leaf curl China virus (TYLCCV) and played role in symptom production (Briddon *et al.*, 2001). The size of DNA β satellites was approximately 1350 bp and it required a helper virus (DNA A of Begomovirus) for replication, encapsidation and transmission from plant to plant (Mansoor *et al.*, 2003; Briddon and Stanley, 2006). Analysis of DNA β sequences has revealed a conserved organization consisting of a single complementary-sense gene (βC1) an adenine-rich region and a satellite-conserved region that carried sequence similarity to the Tomato leaf curl virus (ToLCV) satellite-DNA (Tahir *et al.*, 2011). The DNA β associated with cotton leaf curl disease (CLCuD) encoded a single protein called fiCl which was a pathogenicity determinant (Saeed *et al.*, 2005). A new β-satellite associated with cotton leaf curl Burewala virus infecting tomato in India was also reported which influence symptoms and viral accumulation in the host (Kumar *et al.*, 2013). β-satellites are symptom modulating, pathogenicity determinant and mainly contribute sever symptom expression (Snehi *et al.*, 2015).

Related to the present studies, the available information which was considered to be the relevant was discussed here. However, information have been available on other aspects also but the literature that provided the related and most appropriated information for the study was discussed and considered here.

CHAPTER- III

MATERIALS AND METHODS

The present investigations were carried under laboratory and field conditions at the Department of Plant Pathology and Cotton Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar during the year 2013 and 2014. The materials and methods used during the study were as follows:

3.1 Symptomatology

Fifty tagged plants of cotton were studied for the development of CLCuD symptoms at various plant stages. Plants infected with CLCuV were observed for different symptoms such as, vein thickening (small and main veins), upward leaf curling, formation of enation (small outgrowth at abaxial side of leaf) and stunted growth in case of severe infection. Based on the symptoms and disease severity, the plants were categorized as per of disease scale of 0-6 (Table 3.1 and Table 3.2).

3.2 Epidemiological studies

3.2.1 Experiment 1(a): Role of epidemiological factors on development of CLCuD

The study was carried out to determine the role of various environmental factors involved in disease development under natural epiphytotic conditions. Two cultivars HS-6 (hybrid) and RCH-134 BG-II (Bt), susceptible to CLCuV were used for study purpose. The crop was grown in a plot size 4.05 x 5.40 m^2 in randomized block design with six replications for the each treatment.

Twenty plants in each replication were tagged in each cultivar and weekly disease incidence was recorded on 0-6 scale (Table 3.1) of AICCIP (All India Coordinate Cotton Improvement Project) and disease index/reaction (Table 3.2) was also recorded as per AICCIP scale (Anonymous 2015). Corresponding weather data was obtained from the Department of Agrometeorology, CCS HAU, Hisar (Annexure-I and II) was used for development of regression equation and correlation matrix. The observations recorded under the experiment *viz*., time of disease appearance, disease incidence and index at weekly interval on tagged plants, white fly population on three leaves per plant per week, The apparent infection rate and area under disease progress curve (AUDPC) was calculated. The data were subjected to

statistical analysis by using the statistical software "OPSTAT" provided by the university (http://www.hau.ernet.in/about/opstat.php) and the ANOVA was used to compute the means.

Table 3.1. All India Coordinated Cotton Improvement Project (AICCIP) scale for CLCuD

Disease grade	Disease symptoms
0	Immune
1	Small vein thickening
2	Small vein thickening+main vein thickening + leaf curl
3	Top 1/4th plant part affected (upward/downward curling of leaves)
4	Half (1/2) plant part affected (upward/downward curling of leaves)
5	3/4th plant part affected (upward/downward curling of leaves)
6	Severe stunting of plant (upward/downward curling of leaves)

Table 3.2. Disease index and reaction scale for CLCuD

Disease Index	Reaction
0	Immune/disease free
0.1 - 10	Highly resistant
10.1 - 20	Resistant
20.1 - 30	Moderately Resistant
30.1 - 40	Moderately Susceptible
40.1 - 50	Susceptible
> 50	Highly Susceptible

3.2.2 Experiment 1(b): To study the effect of date of sowing and spacing on incidence of CLCuD

The effect of agronomic practices like variation in date of sowing and spacing were studied during both years on development and CLCuD incidence. Two CLCuV susceptible cultivars *viz.*, HS-6 and RCH-134 BGII were used. Crop was sown at three different date/time *viz.*, Early (1st May), normal (15th May) and late (1st June) as per recommendation of CCS HAU, Hisar. The crop was also sown at three different plant to plant spacing *viz.*, 60, 30 and 15cm and in plot size of 28.35 m^2 (5.40m x 5.25m). Experiment was laid in split plot design with six replications for the each treatment.

The various observation were recorded on disease incidence and disease intensity at weekly interval. Apparent infection rate (*r*) and AUDPC was calculated at weekly also. The agronomical traits *viz.* height of plant (cm), boll number, boll weight (g), seed cotton yield (Kg), ginning out turn (%), seed index (%) and lint index (%) were also observed on ten competitive plants.

3.2.3 Formulas Used

(a) Percent disease incidence

Percent disease incidence (PDI) was calculated by following standard formula:

$$PDI = \frac{\text{Number of diseased plants X 100}}{\text{Total numbers of plants observed}}$$

(b) Disease severity

Percent disease severity (DS) was calculated with formula given by McKinney (1923) as mentioned below:

$$DS = \frac{\text{Sum of all disease ratings X 100}}{\text{Total numbers of rating X maximum disease grade}}$$

(c) Apparent infection rate

Apparent infection rate (AIR) was computed using the formula given by Vanderplank (1963).

$$\text{AIR or } r = 1/(t_2 - t_1) \log \{ x_2 (1 - x_1)/ (x_1 (1 - x_2)\}$$

Where,

t_1 = time of first observation
t_2 = time of next observation
X_1 = disease observation at t_1 time
X_2 = disease observation at t_2 time

(d) Area under disease progress curve (AUDPC)

AUDPC was computed using the formula proposed by Wilcoxson *et al.* (1975):

$$= \sum_{i=1}^{N_i - 1} (y_i - y_{i+1}) \times (t_{i+1} - t_i)/2$$

Where,

N = n^{th} number of entry
y_i = disease index at t_i time
y_{i+1} = disease observation next to i^{th} observation
t_i = time at i^{th}
t_{i+I} = time of next observation

3.3 Biochemical analysis

Four cultivars of cotton (two hybrids and two Bt varieties) *viz.* H 1236, Bunty (resistant cultivars), HS 6 and RCH 134 BG-II as susceptible cultivars were grown at the Cotton Research Area of CCS HAU, Hisar. The cultivars were selected on the basis of their reaction to CLCuV. The biochemical constituents *viz.*, total phenols, sugars (total and reducing), tannins, proteins, gossypol, Chlorphyll-a & b, peroxidase and polyphenol oxidase were analysed using their standard extraction and estimation methods. Leaves of the diseased plants of various grades (0, 2, 4 & 6) were picked at four different plant growth stages (40, 60, 90 and 120 days after sowing) as described in Table 3.3.

Table 3.3. Leaf samples of cotton plants with CLCuD symptoms used for biochemical analysis.

S.N.	Disease grade	Cotton cultivars
1	0	HS-6
2	2	HS-6
3	4	HS-6
4	6	HS-6
5	0	RCH-134 BGII
6	2	RCH-134 BGII
7	4	RCH-134 BGII
8	6	RCH-134 BGII
9	0	H-1236
10	0	Bunty

Fresh and young leaves of cotton plants of uniform age and size were taken from all the four cultivars. Healthy/symptomless (grade-0) leaves from resistant plants, whereas, leaves showing disease grades 2, 4

& 6 from susceptible cultivars were taken for analysis. Symptomless leaves from susceptible cultivars were also taken as control. To remove soil and other insects (eggs/nymphs/adults) from the leaf surface, leaves were first washed with tap water and sun dried for 2-3 days. Thereafter, samples were oven dried at 60^{o} C for 5 days or until complete drying of leaves. The dried leaves were crushed to fine powder for further study of biochemical constituents except for chlorophyll, peroxidase and polyphenol oxidase activity where fresh leaves were used.

3.3.1 Phenol

3.3.1.1 Extraction

Dried cotton leaves were grounded to fine powder with the help of pestle and mortar and weighed to 100mg for the each sample. Powder of each sample was transferred into the test tubes and 5 ml of ethanol (80%) was pipetted in to them. Test tubes were put in to the hot water bath at 80^{o} for 25-30 min and vortexed for proper mixing. After cooling of tubes, the homogenates (samples) were centrifuged at 4000 rpm for 10 minutes. The supernatant of each sample was carefully poured into fresh empty test tubes. After separating supernatant, test tubes containing leaves were again poured with 5ml ethanol. The procedure was repeated with the same original samples and supernatant was pooled in one tube. After collecting the supernatant in one test tube for each sample, tubes were left open overnight to evaporate ethanol of test tubes or until their complete drying. After complete drying of test tube, distilled water (D.W.) was added to make final volume 10 ml in each test tube.

3.3.1.2 Estimation

Total phenol estimation was carried out with the Folin-Ciocalteu's agent. Phenols react with phosphomolybdic acid in Folin-Ciocalteu's reagent in alkaline medium to produce blue coloured molybdenum complex. For estimation, Bray and Thorpe (1954) method was used. In the method two reagents 20% Sodium carbonate solution and Folin's phenol: Distilled water (1:2) were used. From the prepared extract, aliquot (0.5ml) were pipetted out into empty test tubes and 1 ml of refluxed Folin-Ciocalteau reagent was added into each test tube. The solution was kept undisturbed for 3 minutes. After three minutes interval, 2 ml of 20 % Na_2Co_3 was added to each test tube and shaken thoroughly for colour (blue) development. The UV Spectrophotometer was set to zero using blank check by using distilled water in cuvette for each replication. Samples were read at 650nm using spectrophotometer and concentration was calculated by using standard curve of catechol 100 mg / ml.

3.3.2 Sugar

3.3.2.1 Extraction

For extraction of sugar the procedure was common/same as for phenol extraction (section 3.3.1.1) up to the stage of evaporation. The final volume made was 5ml by adding distilled water.

3.3.2.2 Estimation

Total sugar estimation was done as per method of DuBois *et al*. (1956). Oligosaccharides hydrolyzed by concentrated sulfuric acid during the phenol–sulfuric assay and form monomers, namely glucose, fructose and galactose (Sturgeon 1990). Therefore, in the analysis of mono- and oligosaccharides in a plant extract, the intermediate product after acid hydrolysis had mostly a mixture of sugar (glucose, fructose and galactose), that made the principal compounds in the sugar assay.

Two ml of phenol (2%) was added to 0.5 ml of aliquot and 5 ml H_2SO_4 (concentrated) was added rapidly with very care by bottle top dispenser (Zippette ®). After ten minute of color development, the solution was vortexed and allowed to cool at room temperature. Absorbance was measured at 475nm using ultra violet Spectrophotometer (DU 640, Fullerton, USA) against reagent which was blank. Concentration was determined by standard curve prepared using dextrose (10, 50, 100, 200 and 500 mg/ml).

3.3.3 Gossypol

3.3.3.1 Extraction

The grounded cotton leaves, weighing 500 mg for each sample were taken into a conical flask of capacity 25ml and 10 ml of ethyl alcohol (95%) was added into the each flask. Flasks were boiled for 5 minutes and the content was filtered into empty test tubes. The tubes containing the filtrate were then centrifuged at 8000rpm for 15min at a constant temperature of 18^{o}C. The process was repeated with residues in flasks and extracts were combined in to the common test tube for each treatment. The extract was diluted by adding 40% ethanol and pH was adjusted 3.0 by adding 1N HCl. Content of test tubes was mixed with 1.5 ml of diethyl ether at 10^{o}C using a separating funnel. Ether phase was saved and washed two time with distilled water. Ether extract was evaporated *in vacuo* until the tubes got dried and residue was re dissolved in a known volume of 95% ethanol.

3.3.3.2 Estimation

After extraction process the estimation of gossypol was done as per the method of Bell (1967). For this, 0.5 ml of phlorglucinol reagent was added in to 1 ml of aliquots of each sample, followed by

adding 1ml of concentrated HCl. Test tubes were incubated at room temperature for 30 minutes and 80% ethanol was added to make 10ml final volume. Specctrophotometer was adjusted to zero as was done for other biochemical processes and the absorbance of colour was read at 550 nm against a reagent blank. Concentration was determined by making standard curve with gossypol acetate 100 µg / ml.

3.3.4 Tannin

3.3.4.1 Extraction

For the extraction process, grounded cotton leaves (100mg for each treatment) were taken into the plastic centrifuge tubes (oak ridge tube) of capacity 10ml and 5 ml acetone (70 %) was poured into the tubes. Tubes were put in to water bath at 70^{o} C for 25 – 30 min and each tube was vortexed to ensure the proper mixing. The tuber were allowed to cool at room temperature. After cooling, tubes were centrifuged at 4000 rpm for 10 minutes and supernatant was separated out in another empty oak ridge tube. The procedure was repeated twice by adding 5ml acetone (70 %) each time in to tubes with original samples and the final volume was used for the estimation assay.

3.3.4.2 Estimation

Condensed tannin (leucocyanidin equivalent) was estimated by the butanol- HCl method of Porter *et al.* (1986). The concentration of condensed tannin was calculated (% DM) from the formula A_{550nm} x 78.26 x dilution factor/% DM. from the extracted samples collected in oakridge tubes, 0.5 ml extract was taken into the test tubes and 3 ml of butanol – HCl reagent was added, followed by adding 0.1 ml of ferric reagent (Ferric reagent was prepared by adding 2 % ferric ammonium sulphate in 2N HCl). Test tubes containing mixture of extract and reagent were kept in hot water bath at 97-100^{o}C for 1 hour. Reading was on UV Spectrophotometer was taken at 550 nm. Concentration was determined by making standard curve with catechin (120 µg).

3.3.5 Protein

3.3.5.1 Extraction

For each sample, 100mg grounded leaves were taken into a digestion flask of 150 ml capacity. Then 10ml of H_2SO_4 and $HClO_4$ mixed in 4:1 ratio was poured gently along the walls of flask by tilting the flask and were left undisturbed for 24hrs. After 24hrs, the flasks were kept over the hot plate in open area untill the solution lose its colour and became colorless. After complete colour loss, solution was allowed to cool at room temperature. After cooling, little quantity of distilled water (ammonia free) was added initially in the digested material so that the temperature of solution got stabilized. The final volume

of solution was made up to 100ml by adding more distilled water and then the solution was transferred to the distillation apparatus.

3.3.5.2 Estimation

Total protein content from CLCuV infected and healthy leaves was analyzed by the standard method of Kjeldahl as decribed by AOAC (Helrich 1990) as in this method Kjeldahl flask is used. The process included extraction of nitrogen from the prepared solution after extraction process. For nitrogen estimation, 10 ml of 0.01 H_2SO_4 was taken in a 100 ml conical flask and a few drops of indicator (methyl red solution) were added into this. The tip of the condenser was kept dipped in to the solution. Following this, the content of Erlenmeyer flask was transferred into the Kjeldahl flask and attached with a distillation unit. NaOH (40%) and 10 ml digested solution (test solution) were added together in the apparatus (Kjeldahl apparatus). The process results into formation of ammonia gas which was collected in the Erlenmeyer flask of capacity 100ml already containing sulphuric acid into it and at least 40-50 ml of distilled solution was collected by distillation. Tip of condenser was rinsed off with distilled water and solution was titrated against standard acid until the first appearance of violet color (the end point). A blank of reagent with equal volume of distilled water was run and titration volume was subtracted from that of sample.

3.3.6 Chlorophyll-a and chlorophyll-b

The photosynthetic pigments chlorophyll-a & b content from the cotton leaves were extracted and estimated according to the method proposed by Hiscox and Israelstum (1979).

The method involves the estimation of plant pigments without maceration. Freshly picked leaves were kept on a moist filter paper in an icebox, washed with cold distilled water and then chopped to fine pieces. Before taking leaves into the test tubes, 7 ml of dimethyl sulfoxide (DMSO) was poured and 100 mg of the chopped leaf material was then plunged into the tubes. Three test tubes for each treatment were used. The test tubes were then kept in hot water bath at 65°C for 1 hr for the complete leaching of the pigments. The tubes were centrifuged at 4000rpm for 10 minutes. The supernatant was decanted off into the fresh tubes. Thereafter, the final volume of supernatant was made to 10ml by adding DMSO in the tubes and the chlorophyll content was measured immediately. The absorbance of DMSO containing pigments was observed at two different wavelength *viz.* 645 and 663 nm using a UV Spectrophotometer (model DU 640, Fullerton, USA). The amount of total chlorophyll-a and chlorophyll-b was calculated by using the following formula proposed by Arnon (1949):

Total chlorophyll (mg g^{-1})

$$= \frac{(20.2 \times A_{645}) + (8.02 \times A_{663})}{1000 \times W} \times V$$

Chlorophyll-a (mg g^{-1})

$$= \frac{(12.7 \times A_{663}) + (2.69 \times A_{645})}{1000 \times W} \times V$$

Chlorophyll-b (mg g^{-1})

$$= \frac{(22.9 \times A_{645}) + (4.69 \times A_{663})}{1000 \times W} \times V$$

V = Volume of the solution

W = Weight of the sample

3.3.7 Peroxidase enzyme

3.3.7.1 Extraction

Fresh leaves of healthy/diseased cotton leaves were picked and put immediately into an icebox. The fresh samples were brought to laboratory and for each sample 500 mg of leaf tissue was washed in ice cold water. The samples were homogenized manually by adding 4 ml 0.1 M Tris buffer (pH 7.6) and 1 ml 0.1% EDTA in a previously chilled pestle & mortar placed on crushed ice as an abrasive. The homogenate prepared was transferred into the centrifuge tubes (10ml) and were centrifuged at 12,000 rpm for 15 minutes in a refrigerated centrifuge. The supernatant thus obtained was decanted off into glass tubes which was referred as crude extract and stored in deep freezer (-20^{o}C) for the measurement of enzyme activities. Two separate extractions were made for the each treatment/sample.

3.3.7.2 Assay

Peroxidase activity was measured spectrophotometrically by the change in absorbance due to o-dianisidine oxidation in the presence of H_2O_2 and enzyme (Anonymous, 1961). Peroxidase was assayed using O-dianisidine as a substrate to measure peroxidase activity (modified method of Shannon *et al.*, 1966). The reaction mixture contained 3.62 mL of 0.1 M phosphate buffer pH 7.0, 200 µl of 0.1% O-dianisidine, 100 µl of 0.2 (v/v) percent H_2O_2 and 80 µl enzyme extract at 30°C. The blank sample used for standardization contained the same mixture solution without the enzyme extract. The activity of

enzyme was observed with the changes in reading over time on spectrophotometer. One unit of activity was defined as the amount of enzyme that causes an increase of absorbance per minute. The enzymatic reaction was initiated by the addition of H_2O_2 and change in absorbance was followed at 430 nm in spectrophotometer. The enzyme activity was expressed as change in 0.01 absorbance min^{-1} mg^{-1} protein.

3.3.8 Polyphenol oxidase

3.3.8.1 Extraction

Extraction method was same as for perodixase activity (**section 3.3.7.1)**

3.3.8.2 Assay

Similar to peroxidase estimation procedure, supernatant was stored in refrigerator for estimating polyphenol oxidase activities. For the polyphenol oxidase assay modified method by Taneja and Sachar (1974) was used. The reaction mixture was prepared in test tube by adding 1.6 ml 0.1 M Sodium phosphate buffer (pH 7.0), 2.2 ml of 1 percent catechol solution as substrate and 0.2 ml of enzyme extract. The final volume/concentration of reaction mixture obtained was 4.0 ml. Separated blank for each sample was prepared for assay.

Spectrophotometer was set to zero by using blank and the cuvette tube was placed into the spectrophotometer (previously zeroed using a tube with 4ml water and 0.1 ml of the extract). Before starting sample assay, time was noted just before adding enzyme and then enzyme extract was added quickly mixing the contents of the tube. Readings of the absorbance was taken at regular intervals of one minute. The enzyme activity was expressed as change in 0.01 absorbance min^{-1} mg^{-1} protein.

3.4 Molecular studies

CLCuD diseased leaves with distinct symptom variation from different locations in cotton belt of Haryana (Sirsa, Fatehabad, Hisar, Bhiwani and Jind), Punjab (Abohar, Bathinda and Fazilka) and Rajasthan (Hanumangarh and Shriganganagar) were collected (Table 3.4). Total genomic DNA was isolated from diverse CLCuD cotton samples by method of Murry and Thompson (1980) with slight modifications. The cotton genomic DNA was analysed for its quality and concentration by resolving the samples by 0.8% agarose gel electrophoresis. Conditions for amplification for CLCuV specific coat protein genomic DNA were standardized using primers designed from published CLCuV genome sequences. Using total genomic DNAs of diverse CLCuD isolates of Haryana, Punjab and Rajasthan, begomovirus specific polymerization was performed.

3.4.1 Glassware

Glasswares used were of the borosilicate quality and obtained from Borosil Glass Works Ltd., Mumbai or Corning Glass Company, USA. Oven dried (180^{o}C) conical flasks, bottles, test tubes, pipettes,

Petri dishes, beakers, volumetric flasks, Erlenmeyer flasks, measuring cylinders of different sizes (50 ml, 100 ml, 150 ml, 500 ml & 1000 ml) were used for reagents/solutions/media preparations.

3.4.2 Chemicals and Reagents

Chemicals used for DNA extraction and PCR amplification were of molecular biology grade and were obtained from Sigma® Chemicals Co. USA, Life Technologies (India) Pvt. Ltd. Silver staining kit used for SSR analysis was obtained from Promega® Inc. USA. All other chemicals used in the present investigation were of molecular/analytical grade and procured from Hi-Media®, Genetix, Bangalore Genei, E. Merck and SRL, India Ltd.

Table 3.4. List of CLCuD samples used for study collected from Haryana, Punjab and Rajasthan

Sample No.	District	Local place
Haryana		
1	Hisar	CCS HAU
2		Chaudharywas
3		Kaimari
4		Aryanagar
5		Matersyam
6		Barwala
7		Uklana
8		Adampur
9		Agroha
10		Bodha Hoshnak
11		Moda Khera
12		Channi Badi
13		Balsamand
14		Hansi
15	Fatehabad	Fatehabad
16		Hamjapur
17		Basin
18		Bhattukalan
19		Ding
20		Ratia
21		Kawalgarh
22		Tohana
23		Nathusari kalan
24		Bhuna
25	Sirsa	Sirsa
26		Odhan
27		Kalanwali
28		Jagmalwali
29		Ellanabad

30		Rania
31		Jeevan Nagar
32		Mandi dabwali
33		Abubshahar
34		Dudhiawali
35	Bhiwani	Bhiwani
36		Bawanikhera
37		Badhra
38		Siwani
39		Jind
40		Narwana
41		Danoda Kalan
42		Unchana
Punjab		
43	Bathinda	Bathinda
44		Pathrala
45		Kishanpur
46		Malaut
47		Talwandi Sabo
48		Rori
49	Fazilka	Fazilka
50		Ganjuana
51		Kui Khera
52		Nihalkhera
53		Chuvadiawali
54		Panniwala
55	Abohar	Abohar
56		Kabarwala
57		Pakki Tibbi
58		Khuiya sarbar
59		Kallar khera
Rajasthan		
60	Sri Ganganagar	Sri Ganganagar
61		Sadhuwali
62		Sadul sahar
63		Lalgarh Jatan
64		Mahiyanwali
65		Jogiwala
66	Hanumangarh	Hanumangarh
67		Pakka Sahrana
68		Rodawali

3.4.3 Buffers and solutions

The chemicals/reagents were used for preparing buffers and solutions are mentioned in Table 3.5 and were sterilized as per the need.

Table 3.5 Composition and preparation of important solutions and buffers

Reagent	Composition and method of preparation
DNA Extraction Buffer	100 mM Tris (pH 8.0), 20 mM EDTA (disodium salt. pH 8.0), 1.4 M Sodium chloride, 2% CTAB and 0.2 M β-mercaptoethanol.
0.5 M EDTA	186.l g Disodium ethylene-diamine tetra acetate. $2H_2O$ was dissolved in 800 ml, distilled water by stirring vigorously and pH was adjusted to 8.0 with NaOH pellets. Solution was sterilized by autoclaving.
Ethidium bromide (10 mg/ml)	One gram ethidium bromide was added to 100 ml of water and stirred vigorously on a magnetic stirrer for several hours to ensure that the dye has dissolved. The container was wrapped in aluminum foil and stored at 4ºC.
Gel loading buffer/dye	Sucrose 4.0 g, Bromophenol blue 0.025 g and Xylene cyanol 0.025 g were dissolved in water and volume was made to 10 ml. It was stored at 4ºC.
5M NaCl	292.1 g of NaCl was dissolved in 800 ml of distilled water and volume adjusted to 1L. Solution was sterilized by autoclaving.
I M Tris	121.1 g of Tris base was dissolved in 800 ml of distilled water and the pH was adjusted to 8.0 by adding concentrated HCl. The volume was made up to 1L and sterilized by autoclaving.
TE buffer	10 mM Tris (pH 8.0), 1 mM EDTA (disodium salt, pH 8.0)

3.4.4 Amplification of Cotton Leaf Curl Virus coat protein specific gene sequences

Polymerase chain reaction technique was used to amplify CLCuV coat protein gene from genomic DNA of different leaf samples of cotton plants. For this purpose three pairs of coat protein gene specific primers of Cotton Leaf Curl Virus (CLCuV) were got synthesized from Bioserve Pvt. Ltd. Hyderabad and used in the present study (Table 3.6).

3.4.5 Extraction of genomic DNA

Genomic DNA of 80 cotton leaves sample was isolated using CTAB method of Saghai-Maroof *et al.* (1984) from leaf tissue showing CLCuD symptoms. Leaf samples were cut into small

pieces and ground into fine powder using liquid nitrogen in a sterilized pestle and mortar. Leaf tissue powder was transferred into a 50 ml centrifuge tube. To this, 15 ml of preheated CTAB buffer was added and the samples were thoroughly mixed by inverting the tubes several times. The tubes were incubated at 65^{o}C in water-bath for 1 hour and 30 minutes and inverted a few times intermitently for mixing of contents during incubation.

After incubation the samples were cooled to room temperature and then, 15 ml solution of chloroform & isoamyl alcohol in ratio of (24:1) was added. Samples were again mixed thoroughly by gently inverting the tubes several times and centrifuged for 15 min at 10,000 rpm and the aqueous layer was transferred into fresh pre sterilized, labelled centrifuge tubes.

Equal volume of ice-cold isopropanol was subsequently added to precipitate the DNA and the tubes were kept undisturbed for 15 minutes. The DNA was then spooled out and put into 1.5 ml Eppendorf centrifuge tubes. After that DNA was washed in 70% ethanol two times and left for 20minutes. DNA was then air-dried overnight at room temperature and subsequently dissolved in appropriate volume of TE buffer. Samples were stored at -20^{o}C till further use.

Table 3.6 List of molecular markers

S.N.	Id/Name	Sequence (5'-3')	Melting Temp (^{o}C)	Annealing temperature (^{o}C)		Reference
1	CtLCV#33 F	TAATATCAATTCGTTACAGAG	45	43.5	Lucknow	Kumar *et al.*, 2010
	CtLCV#33 R	AATTATGTCGAAGCGAGCTG	50			
2	CtLCV#45 F	GCCATGTCAGGAAGCCAAC	53	49.0	Pakistan	Khan and Ahmad, 2005
	CtLCV#45 R	GGATTAGAGGCATGACGTACAT	53			
3	CtLCV#55 F	GGTCCCCTCCACTAAATCAT	52	49.0	Dharwad	Reddy, 2006
	CtLCV#55 R	CAGTTGGTTACAGAATCGTAGAAG	54			

3.4.6 Removal of phenolics

Cotton leaves contains phenolic compounds and showed precipitation with the nucleic acid. Non removal of phenolics would lead to the degradation of DNA and can also hinder in PCR amplification. A PVP concentration of 2% was added and after the addition in the tubes (containing DNA), the contents were mixed by gentle inversion for 5 min. After mixing, the tubes were centrifuged for 10 min. at

12000rpm speed. Centrifugation resulted in separation of two layers. The upper aqueous layer containing DNA was transferred into fresh 1.5ml eppendorf tube.

3.4.7 RNAse treatment

For purification, 1 µl RNAse solution was added to the tube (50 µg/ml) and the mixture was incubated for 3 hour and 30 minute at 37ºC in a water bath. After incubation, DNA was extracted with chloroform : isoamyl alcohol (24:1) by centrifuging the tubes at 10,000 rpm for 5 minutes at room temperature. DNA was precipitated with 2 volume of ice-cold absolute alcohol and pellet of DNA was obtained by centrifuging the tubes at 5000 rpm for 10 minutes. The pellet was washed with fresh 70 per cent ethanol and then air dried. Dried pellet was dissolved in appropriate volume of TE buffer and stored at -20ºC.

3.4.8 Quantitative and qualitative estimation of genomic DNA

When DNA was completely dissolved in TE, its concentration and quality were checked by running 2µl of DNA sample on Agarose gel electrophoresis (0.8%). A standard marker was used to check the quality of genomic DNA Gel casting plate was washed, air-dried and its ends were sealed with tape. Agarose was melted in 10x TBE buffer and ethidium bromide (5µg/ml) was added. Gel solution was then poured into gel casting plate, an appropriate comb was inserted. After setting of gel, sealing tapes were removed from both the ends. Gel plate was placed in the electrophoresis chamber and submerged using 1 X TBE buffer and comb was removed gently.

Samples were prepared by adding 6x loading dye. Samples were loaded in the wells and electrophoresis was carried out in constant voltage (75V) mode. The gel was visualized in a UV transilluminator. The DNA concentrations were estimated by visual assessment of band intensity in comparison with *Lambda* (λ). DNA of known concentration were run on electrophoresis using 0.8% agarose gel. The quality of DNA samples was estimated as good (if sharp and discrete band appeared), sheared (if smear was there) and improper dissolution (if thin lane appeared).

3.4.9 Agarose Gel Electrophoresis

PCR amplified DNA products were resolved by submerged horizontal electrophoresis in 2.5 % (w/v) agarose gels. Agarose was melted in 10x TBE buffer and ethidium bromide (5µg/ml) was added. Gel solution was then poured into gel casting plate, an appropriate comb was inserted. After setting of gel, comb was removed gently then sealing tapes were removed from both the ends.

Gel plate was placed in the electrophoresis chamber and submerged, using 10x TBE buffer. DNA samples were prepared by adding 2X loading dye and pulse centrifuged for proper mixing. Samples were loaded in the wells and electrophoresis was carried out at constant voltage (3 v/cm of gel) until dye

migrated to other end of the gel. PCR amplified products were viewed under UV light (350 nm) fluorescence and photographs were taken by gel documentation system. Stock solution for gel electrophoresis are given in (Table 3.7).

Table 3.7 Stock Solutions of gel electrophoresis

10X TBE buffer	
Tris	108.0 g
Boric acid	55.0 g
0.5 M EDTA (pH 8.0)	40 ml
Final volume	1000 ml
6X loading dye	
Sucrose	4.0 g
Bromophenol blue	0.025 g
Xylene cyanol	0.025 g
Final Volume	10 ml

Loading dye solution was stored at 4°C in the refrigerator.

CHAPTER-IV

EXPERIMENTAL RESULTS

The results obtained in present studies from experiments conducted under field and laboratory conditions have been explained and presented in different heads as follows:

4.1 Symptomatology

4.2 Epidemiological studies

4.2.1 Role of epidemiological factors on development of CLCuD

4.2.2 Effect of date of sowing and plant spacing on disease development

4.3 Biochemical studies

4.4 Molecular diversity/variability studies

4.1 Symptomatology

Cotton leaf curl disease (CLCuD) was caused by cotton leaf curl virus (CLCuV) appeared in the field under natural epiphytotic conditions during the two years of study. A series of symptoms were appeared on cotton on two susceptible cultivars HS-6 and RCH 134 BG-II. Initially, the disease appeared as the infected leaves started to show small and main vein thickening during early phase of incidence (Plate 4.1a) and later on the diseased leaves showed upward curling (Plate 4.1b). As the disease advances, the infected leaves became cup like structures. Severely infected plants exhibited the formation of leaf like structure underside of cotton leaves (Plate 4.1c) which indicated confirmatory symptom of CLCuD due to physiological changes in the plant. Further, as the plant reached near maturity, severely infected plants showed stunted growth and resulted in reduction in number of bolls. Finally, the CLCuD infected plant gave a bushy appearance as compared to the healthy plant (Plate 4.1d).

4.2 Epidemiological studies

4.2.1 Role of epidemiological factors on the development of CLCuD

4.2.1.1 Disease appearance, percent incidence and progression

The disease appearance, incidence and progression was recorded on fifty selected plants in the field for each cultivar. Weekly observations on disease development revealed that first appearance of disease was observed on 21st June (3rd week of crop stage) in both the cultivars which reached maximum (100%) at 9th week of crop stage after infection during 2013-14 (Table 4.1). Whereas, during 2014-15, disease appeared on 30th June (5th week of crop stage) and reached to maximum (100%) during 10th week of crop stage (Table 4.2). the varietal behavior of CLCuD was also recorded and revealed that both the cultivars were susceptible to CLCuD and there were no variations in the development of CLCuD.

Plate 4.1 (a) Vein thickening after CLCuV infection in cotton plant, (b) upward curling of infected leaves, (c) enation at abaxial side of leaf and (d) bushy plant under severe infection.

The progression of disease on two cultivars was observed on weekly basis. The data presented in Table 4.1 revealed that after appearance of disease on 23 DAS, the progression of disease reached maximum 38.4% up to 37 DAS in cultivar HS-6. Whereas, it reached maximum 60.3% up to 44 DAS in RCH 134 BG-II in year 2013. Thereafter, the disease progression declined and plants were fully infected after 58 days of sowing.

Similarly, observations recorded during 2014 revealed that after appearance of disease on 30 DAS, disease progression reached maximum 33.0% on HS-6 and 34.4% on RCH 134 BG-II up to 51 DAS. Thereafter, the plants were fully infected and not much disease progression was recorded. The data revealed that, the disease appeared bit earlier (23DAS) during 2013, whereas, it appeared at 30 DAS during 2014. However, the progression was maximum during 2014 as compared to 2013.

Perusal of data from Table 4.1 and 4.2 revealed that, the disease initiated around 4th week of crop age during 2013 and around 5th week during 2014 and reached maximum within 9-10 week of crop age in both the cultivars. It indicated that there was no such difference in hybrid as well as Bt cotton in respect to CLCuD infection and multiplication.

Data presented in Fig 4.2 depicted that after disease appearance, the maximum progression of disease on cv. HS-6 (38.4%) was observed at 35 DAS and it reached maximum 60.3% at 42 DAS on cv. RCH 134 BG-II. Thereafter, progression was slow in both the cultivars. The disease progression during 2014 presented in Fig 4.2 depicted that maximum disease progression was reached at 49 DAS in both the cultivars. The observation revealed that maximum disease progression was recorded when temperature (maximum) was in the range of 35.5-38.7 ^{o}C during 2013. Whereas, it was ranged between 40.5 to 38.5^{o}C in 2014. During the disease progression period, Temperature (minimum) was ranged between 25.0 to 28.4 ^{o}C for HS-6 and 25.0 to 27.5 ^{o}C for RCH 134 BG-II in 2013. Whereas, in 2014 the range of Temperature (minimum) was observed between 27.3 to 28.2 ^{o}C.

Relative humidity (morning) was in the range of 81.1-78.1% and relative humidity (evening) was in the range of 73.4-55.0% during 2013. During, 2014 relative humidity (morning) was ranged from 66.0 to 64.0% and relative humidity (evening) was ranged from38.0 to 43.0%. During 2013 occurrence of rainfall (97.3mm) was only at 21 DAS during 2013, whereas, during 2014, 37.5mm, 10.6mm and 14.5mm of rainfall was observed on 21, 28 and 35 DAS, respectively. Sunshine hours (BSS) were 4.0 to 6.9 during 2013 and 7.3 to 4.8 during 2014.

Table 4.1. CLCuD disease incidence and periodical disease progression on two cotton cultivars during 2013

Crop age		HS-6*		RCH 134 BG-II**	
Days after sowing	**Weeks after sowing**	**Disease incidence (%)**	**Disease Progression (%)**	**Disease incidence (%)**	**Disease Progression (%)**
23	**4**	1.5	1.5	1.2	1.2
30	**5**	17.3	15.8	13.3	12.1
37	**6**	55.7	38.4	24.0	10.7
44	**7**	86.7	31.0	84.3	60.3
51	**8**	97.6	10.9	95.3	11.0
58	**9**	100.0	2.4	100.0	4.7
65	**10**	100.0	0.0	100.0	0.0
72	**11**	100.0	0.0	100.0	0.0
79	**12**	100.0	0.0	100.0	0.0
86	**13**	100.0	0.0	100.0	0.0
93	**14**	100.0	0.0	100.0	0.0
100	**15**	100.0	0.0	100.0	0.0
107	**16**	100.0	0.0	100.0	0.0
114	**17**	100.0	0.0	100.0	0.0
121	**18**	100.0	0.0	100.0	0.0

*hybrid cotton, **Bt cotton

Table 4.2. CLCuD disease incidence and periodical disease progression on two cotton cultivars during 2014

Crop age		HS-6*		RCH 134 BG-II**	
Days after sowing	**Weeks after sowing**	**Disease incidence (%)**	**Disease Progression (%)**	**Disease incidence (%)**	**Disease Progression (%)**
30	**5**	6.8	6.8	12.3	12.3
37	**6**	22.5	15.7	27.8	15.5
44	**7**	49.3	26.8	59.3	31.5
51	**8**	82.3	33.0	93.7	34.4
58	**9**	95.7	13.4	98.4	4.7
65	**10**	100.0	4.3	100.0	1.6
72	**11**	100.0	0.0	100.0	0.0
79	**12**	100.0	0.0	100.0	0.0
86	**13**	100.0	0.0	100.0	0.0
93	**14**	100.0	0.0	100.0	0.0
100	**15**	100.0	0.0	100.0	0.0
107	**16**	100.0	0.0	100.0	0.0
114	**17**	100.0	0.0	100.0	0.0
121	**18**	100.0	0.0	100.0	0.0

*hybrid cotton, **Bt cotton

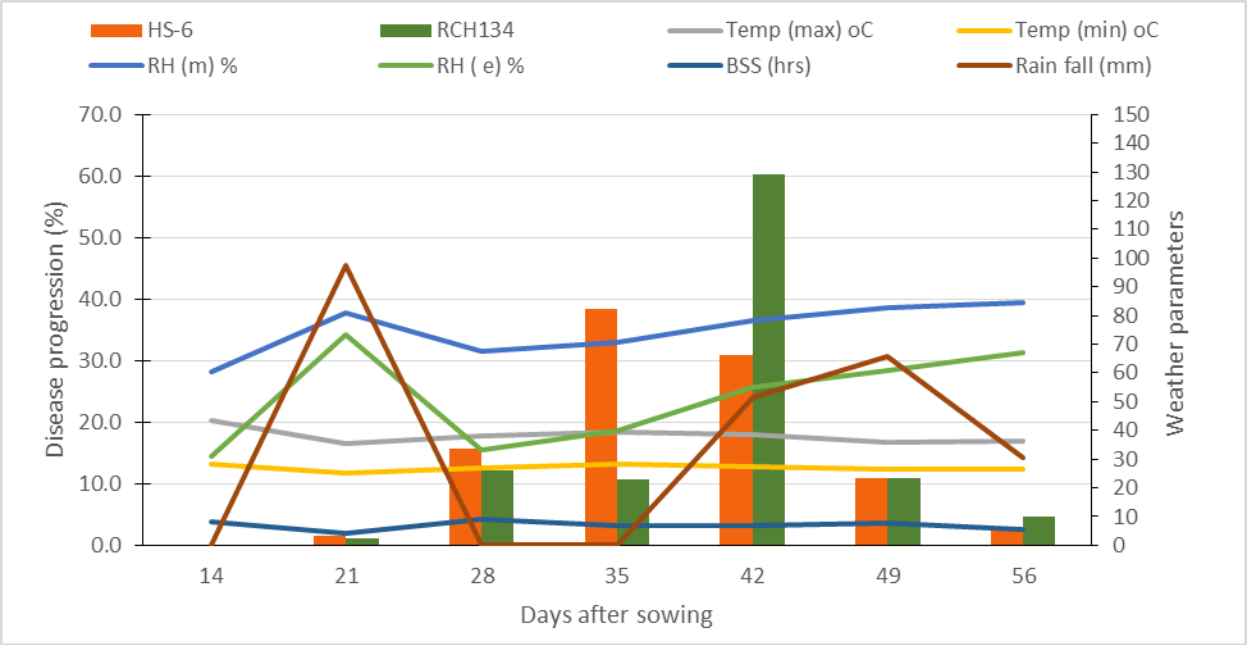

Fig 4.1 Disease progression (%) of CLCuD on two cotton cultivars in relation to weather parameter during 2013

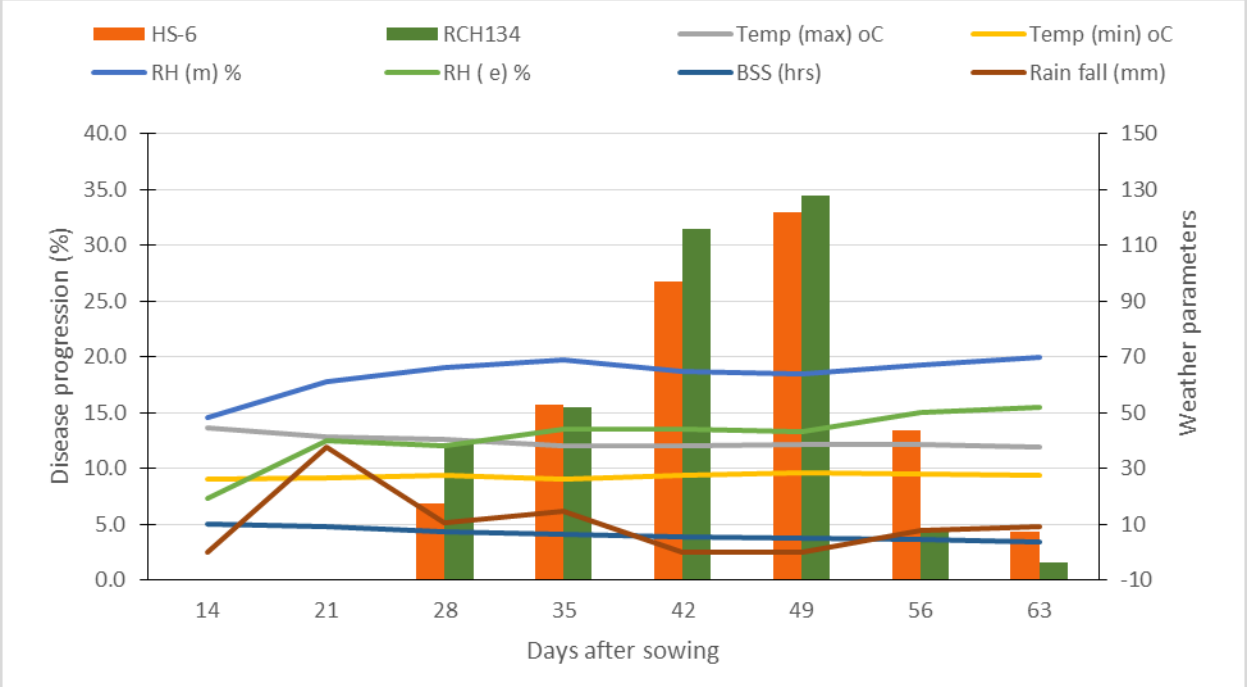

Fig 4.2 Disease progression (%) of CLCuD on two cotton cultivars in relation to weather parameter during 2014

4.2.1.2 Development of prediction model

The observed values of prediction equation for CLCuD were compared with predicted values by statistical analysis. The results revealed that the predicted values showed almost similar trend as of the original values on both the varieties during both the years (Fig 4.3).

The data revealed that observed values of HS-6 were very close to the expected values during 2013 and showed around 97% similarity. The observed and expected values showed least similarity 92% in RCH 134 BG-II during 2013. Whereas, observed and expected disease progression showed about 95% closeness on both the cultivars during 2014.

4.2.1.3 Disease severity, area under disease progress curve (AUDPC) and apparent infection rate (r)

4.2.1.3.1 Disease severity

The disease severity of CLCuD on two cotton varieties was recorded for the two years. The results presented in Table 4.3 revealed that the severity of the disease was varied from 1.03 to 72.55% on HS-6 and 1.30 to 77.05% on RCH 134 BG-II, being maximum at 18 weeks after sowing in both the cultivars during 2013. Whereas, data presented in Table 4.4 revealed that disease severity was varied from 1.10 to 57.10% on HS-6 and 2.00 to 57.30% on RCH 134 BG-II, being maximum at 18 weeks after sowing in both the cultivars during 2014.

Table 4.3. CLCuD disease severity (DS), area under disease progress curve (AUDPC) and apparent infection rate (AIR) on two cotton cultivars during 2013.

Crop age		HS-6			RCH 134 BG-II		
Days	**Weeks**	**DS (%)**	**AUDPC**	**AIR**	**DS (%)**	**AUDPC**	**AIR**
23	**4**	1.03	-	-	1.30	-	-
30	**5**	6.30	25.66	0.72	8.37	33.85	0.77
37	**6**	9.01	53.59	0.23	11.58	69.83	0.34
44	**7**	12.66	75.85	0.18	16.02	96.60	0.41
51	**8**	18.25	108.19	0.12	20.11	126.46	0.24
58	**9**	24.76	150.54	0.07	26.30	162.44	0.10
65	**10**	32.03	198.77	0.06	38.45	226.63	0.05
72	**11**	38.62	247.28	0.07	46.60	297.68	0.05
79	**12**	43.26	286.58	0.05	51.13	342.06	0.13
86	**13**	47.74	318.50	0.05	56.10	375.31	0.05
93	**14**	52.92	352.31	0.06	73.28	452.83	0.03
100	**15**	57.64	386.96	0.07	66.04	487.62	0.05
107	**16**	62.33	419.90	0.05	70.29	477.16	0.06
114	**17**	68.76	458.82	0.04	76.84	514.96	0.04
121	**18**	72.55	494.59	0.03	77.05	538.62	0.04

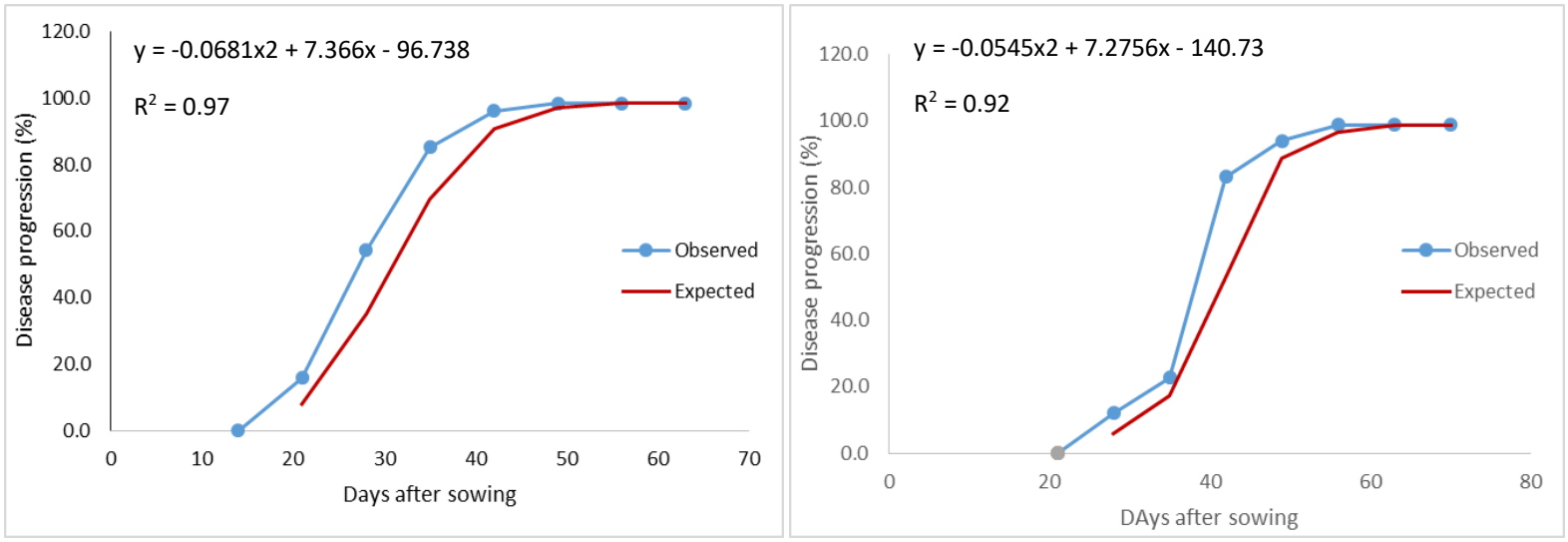

Fig 4.3 Predicted and observed disease progression on two cotton cultivars during 2013 and 2014

4.2.1.3.2 Area under disease progress curve

Area under disease progress curve (AUDPC) was calculated as per the formula of Wilcoxon *et al*. (1975) on both the cultivars. The perusal of data in Table 4.3 revealed that AUPDC was varied from 25.66 to 494.59 on HS-6 and 33.85 to 538.62 on RCH 134 BG-II, being maximum at 18 weeks after sowing during 2013. Similarly, data presented in Table 4.4 revealed that AUDPC during 2014 was varied from 10.85 to 374.85 on HS-6 and from 29.40 to 388.64 on RCH 134 BG-II, being maximum at 18 during 2014.

4.2.1.3.3 Apparent infection rate

The apparent infection rate (AIR) of CLCuD on the both cultivars was also calculated as per the formula of Vanderplank (1963). The perusal of data in Table 4.3 revealed that maximum apparent infection rate was observed in the 5th week of crop age on both the cultivars during 2013. The apparent infection rate was varied from 0.03 to 0.72 on HS-6 and 0.03 to 0.77 on RCH 134 BG-II.

Similarly, data presented in Table 4.4 revealed that apparent infection rate on HS-6 was varied from 0.04 to 0.94 and 0.04 to 0.84 on RCHC 134 BG-II during 2014. Maximum apparent infection rate was observed in the 7th week of crop age on HS-6 (0.94) whereas, in the 6th week apparent infection rate was observed being maximum on RCH 134 BG-II (0.84).

Table 4.4. CLCuD disease severity (DS), area under disease progress curve (AUDPC) and apparent infection rate (AIR) on two cotton cultivars during 2014

Crop age		HS-6			RCH 134 BG-II		
Days	**Weeks**	**DS (%)**	**AUDPC**	**AIR**	**DS (%)**	**AUDPC**	**AIR**
30	**5**	1.10	-	-	2.00	-	-
37	**6**	2.00	10.85	0.83	6.40	29.40	0.84
44	**7**	7.50	33.25	0.94	13.00	67.90	0.75
51	**8**	12.18	68.88	0.16	16.55	103.43	0.22
58	**9**	17.83	105.04	0.08	21.28	132.41	0.09
65	**10**	24.70	148.86	0.08	24.90	161.63	0.06
72	**11**	29.80	190.75	0.06	32.80	201.95	0.06
79	**12**	33.00	219.80	0.05	37.10	244.65	0.07
86	**13**	36.10	241.85	0.06	40.40	271.25	0.04
93	**14**	38.60	261.45	0.12	43.70	294.35	0.09
100	**15**	40.90	278.25	0.11	48.00	320.95	0.08
107	**16**	43.70	296.10	0.07	50.70	345.45	0.07
114	**17**	50.00	327.95	0.07	53.74	365.54	0.05
121	**18**	57.10	374.85	0.04	57.30	388.64	0.05

4.2.1.3.4 Regression equations for progression of CLCuD

The disease severity was subjected to multiple regression in relation to weather variable. The multiple regression analysis of data in relation to weather variables (independent variables) revealed that all the weather variables played important role in disease development. The data presented in Table 4.5 revealed that during year 2013, R^2 value observed was 0.88 and 0.87 for HS-6 and RCH 134 BG-II, respectively. Whereas, during 2014, R^2 value was 0.70 and 0.69 for HS-6 and RCH 134 BG-II, respectively. Higher R^2 values indicated that all the weather variables have contributed 88% in disease development during 2013. Whereas, 70% role of weather variables in disease progression was observed during 2014.

Table 4.5 Multiple regression equations for the progression of CLCuD on two cotton cultivars in relation to weather parameters during 2013 and 2014

Year	Cultivars	Regression equation	R^2
2013	HS-6	$Y = -684.75 + 7.095X_1 - 4.014X_2 + 5.688X_3 + 0.974X_4 + 13.636X_5 - 0.277X_6$	0.88
	RCH 134 BG-II	$Y = -488.502 + 3.510X_1 - 3.977X_2 + 4.993X_3 + 0.850X_4 + 12.815X_5 - 0.311X_6$	0.87
2014	HS-6	$Y = 323.595 - 11.348X_1 + 2.765X_2 - 0.496X_3 + 1.801X_4 + 7.895X_5 - 0.639X_6$	0.70
	RCH 134 BG-II	$Y = 402.562 - 12.770X_1 + 3.185X_2 - 1.004X_3 + 1.911X_4 + 7.538X_5 - 0.652X_6$	0.69

Where, $X_1 = T_{max}$(maximum temperature), $X_2 = T_{min}$(minimum temperature), $X_3 = RH_{mor}$ (morning relative humidity), $X_4 = RH_{eve}$(evening relative humidity), X_5 = BSS (bright sunshine hours), X_6 = RF (rainfall)

Stepwise regression was also computed to understand the role of individual meteorological factors. Perusal of data in Table 4.6 revealed that R^2 value varied from 0.00 to 0.59 for HS-6 and 0.00 to 0.58 for RCH 134 BG-II during 2013. It was revealed from the data that among the various weather variables, two factors *viz.* T_{max} and RH_{mor} played important role in the progression of disease. The regression equation developed and presented in Table 4.6 revealed that T_{max} (X_1) and RH_{mor} (X_3) contributed more than 50% in the development of disease during 2013, whereas, other factors (independent) showed less contribution in the disease development. Similarly, during 2014, T_{max} (X_1) and RH_{mor} (X_3) contributed nearly 61 and 58% on HS-6, whereas, up to 61 and 55% respectively on RCH 134 BG-II. Role of BSS and RF in the development of disease has been observed comparatively low and showed very low R^2 values during both the years (Table 4.6).

Table 4.6 Stepwise regression equations for the progression of CLCuD on two cotton cultivars in relation to weather parameters during 2013 and 2014

Characters	Regression equations	R^2
	2013	
HS-6	$y = 445.257-10.377X_1$	0.52
	$y = 403.181 - 12.688X_2$	0.24
	$y = -207.186 + 3.435X_3$	0.59
	$y = -9.985 + 1.441X_4$	0.26
	$y = 54.814 + 2.435X_5$	0.01
RCH 134 BG-II	$y = 471.762-11.189X_1$	0.55
	$y = 438.366 - 14.139X_2$	0.28
	$y = -218.406 + 3.540X_3$	0.58
	$y = -14.374 + 1.472X_4$	0.25
	$y = 49.127 + 2.854X_5$	0.01
2014		
HS-6	$y = 522.738-12.149X_1$	0.61
	$y = 355.282 - 10.949X_2$	0.11
	$y = -135.956 + 2.757X_3$	0.58
	$y = -46.949 + 2.384X_4$	0.43
	$y = 91.078-3.574X_5$	0.03
	$y = 65.792 + 0.042X_6$	0.03
RCH 134 BG-II	$y = 517.201-11.948X_1$	0.61
	$y = 317.881 - 9.455X_2$	0.08
	$y = -126.228 + 2.651X_3$	0.55
	$y = -43.783 + 2.36X_4$	0.44
	$y = 99.14-4.447X_5$	0.05
	$y = 68.228 + 0.007X_6$	0.02

X_1 = Temperature- maximum (T_{max})
X_2 = Temperature- minimum (T_{min})
X_3 = Relative humidity- morning (RH_{mor})
X_4 = Relative Humidity- evening (RH_{eve})
X_5 = Bright sunshine hours (BSS)
X_6 = Rainfall (RF)

The data presented in Table 4.6 revealed that T_{min} and RH_{eve} contributed about 24 and 26% respectively for disease development on cv. HS-6, whereas, these factors contributed up to 28 and 27%, respectively for cv. RCH 134. Perusal of data in Table 4.6 revealed that during 2014 also RH_{eve} contributed 44% in the disease progression in both cultivars. The T_{min} was observed non favourable during this year hence contributed very low in progression of disease. So disease progression was less during this year.

4.2.1.3.5 Correlation of CLCuD with weather variables

Correlation matrix for the progression of CLCuD in relation to weather parameters was calculated for both cultivars. The data presented in Table 4.7 revealed that T_{max} showed negative but significant correlation in the disease progression in both the cultivars. Whereas, RH_{mor} and RH_{eve} showed significant positive correlation on both the cultivars during both the years. Two variables T_{max} and T_{min} showed significant negative correlation on disease development on RCH 134 BG-II during 2013 only.

Table 4.7 Correlation matrix for the progression of CLCuD in relation to various meteorological parameters in HS-6 during 2013

CLCuD	2013		2014	
	HS-6	RCH 134 BG-II	HS-6	RCH 134 BG-II
T_{max} (X_1)	-0.719^{**}	-0.745^{**}	-0.784^{**}	-0.782^{**}
T_{min} (X_2)	-0.490^{NS}	-0.525^{*}	-0.330^{NS}	-0.290^{NS}
RH_{mor} (X_3)	0.767^{**}	0.760^{**}	0.760^{**}	0.742^{**}
RH_{eve} (X_4)	0.514^{*}	0.504^{*}	0.657^{**}	0.661^{**}
BSS (X_5)	0.101^{NS}	0.114^{NS}	-0.170^{NS}	-0.215^{NS}
RF (X_6)	0.164^{NS}	0.152^{NS}	0.020^{NS}	0.004^{NS}

Perusal of data in Table 4.7 revealed that three weather variables T_{max}, $RH_{morning}$ and $RH_{evening}$ have shown significant correlation with the disease progression in both the cultivars during both the years. Data revealed that T_{min} had non significant correlation in both the cultivars during 2014 (Table 4.7).

4.2.1.3.6 Whitefly population and disease incidence

The studies on white fly population were also recorded on both the cultivars during both the years. The weekly observation of white fly number throughout the crop growth stages was recorded. White flies were counted during early morning hours before sunrise (Plate 4.2).

The data presented in Table 4.8 showed that the whitefly population was low during 2013 and higher in 2014. The data presented in Table 4.8 revealed that both the cultivars HS-6 and RCH 134 BG-II had attack of vector white fly. White fly was appeared on the crop at 2 weeks after sowing (14 DAS) during 2013 in a total span of 19 weeks. The white fly population was ranged from 1.2 to 162.1 on HS-6

and 1.7 to 168.2 on RCH 134 BG-II during 2013. Maximum number of white fly was observed at 19 weeks after sowing in both the cultivars. Whereas, perusal of data in Table 4.8 revealed that the white fly appeared at 3 weeks after sowing during 2014. The population was ranged from 4.6 to 303.4 on HS-6 and 5.8 to 310.0 on RCH 134 BG-II during 2014. Data showed that maximum white fly population was observed at 17 weeks after sowing in both the cultivars. At the end of the season at 133 DAS (19 weeks), the final population of whitefly reached to 289.2 and 304.5 in HS-6 and RCH 134 BG-II respectively (Table 4.8).

The trend of white fly population from Fig 4.3 revealed that white fly number was increased gradually during 2013. The white fly population reached maximum (162.1 and 168.2) on HS-6 and RCH 134 BG-II, respectively at 18th weeks after sowing. However, little fluctuation in white fly population was observed between 42 to 56 DAS.

Fig 4.4 also revealed that rapid fluctuation in white fly population was observed during 2014. White fly population was increased up to 49 DAS and thereafter, a downfall in white fly number was observed up to 98 DAS in both the cultivars. Whereas, white fly population was increased rapidly 93.9 to 276.3 on HS-6 and 101.8 to 293.7 on RCH 134 BG-II within a week (98 to 105 DAS). Thereafter, very little increase in white fly number was observed (Fig 4.4).

Table 4.8 White fly population in two cotton cultivars during 2013 and 2014

Days after sowing	Weeks after sowing	2013		2014	
		HS-6	RCH134 BG-II	HS-6	RCH134 BG-II
14	**2**	1.2	1.7	0.0	0.0
21	**3**	8.4	6.6	4.6	5.8
28	**4**	15.1	14.4	18.4	15.6
35	**5**	23.6	25.4	46.2	50.2
42	**6**	33.3	38.3	66.5	78.4
49	**7**	34.6	37.2	120.0	131.0
56	**8**	69.9	76.9	107.8	110.0
63	**9**	65.7	69.0	103.4	109.1
70	**10**	65.9	69.9	116.4	119.1
77	**11**	84.3	87.8	112.2	121.7
84	**12**	92.9	94.8	107.4	114.5
91	**13**	107.4	112.7	98.5	103.0
98	**14**	117.2	120.2	93.9	101.8
105	**15**	120.1	123.2	276.3	293.7
112	**16**	127.0	134.3	303.4	310.0
119	**17**	138.1	155.9	291.8	296.9
126	**18**	157.2	163.8	293.7	303.4
133	**19**	162.1	168.2	289.2	304.5

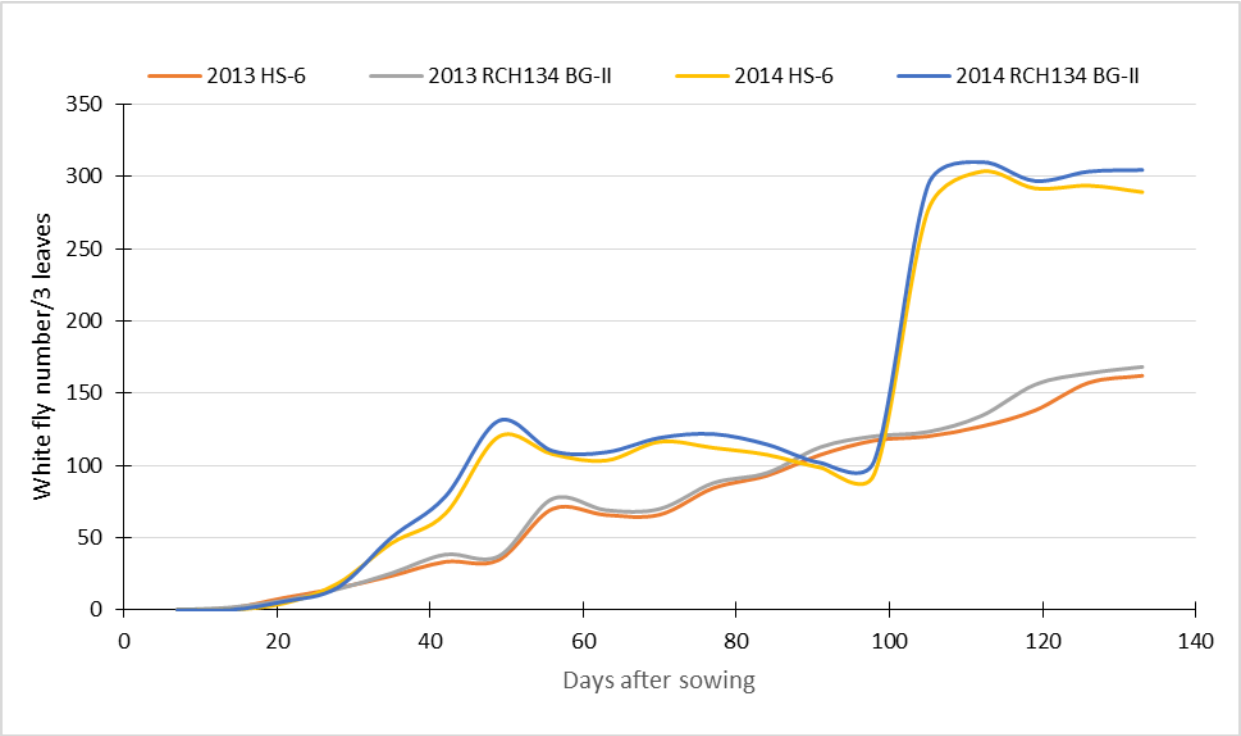

Fig 4.4 White fly population on cotton cultivars HS-6 and RCH 134 BG-II during 2013 and 2014

Plate 4.2 White fly population at abaxial side of cotton leaf

The perusal of data from Table 4.8 revealed that during 2013, white fly population showed little fluctuation in population. White fly population showed a little sudden increase in population from 34.6 to 69.9 on HS-6 and 37.2 to 76.9 on RCH 134 BG-II at 7^{th} to 8^{th} weeks after sowing. Thereafter, with the little decline in number, whitefly population showed sudden rise to 92.9 and 94.8 on HS-6 and RCH 134 BG-II, respectively from 10^{th} to 12^{th} weeks after sowing. The weather variable studied during the period showed that T_{max} was changed from 35.7 – 36.1^{o}C during 7^{th} to 8^{th} week and 31.7 – 34.6 ^{o}C during 10^{th} to 12^{th} week. Similarly, T_{min} showed little change from 26.5 – 26.4 ^{o}C during 7^{th} to 8^{th} week and 25.4 – 26.9 ^{o}C during 10^{th} to 12^{th} week. Increase in RH_{mor} was observed 82.6 to 84.4% during 7^{th} to 8^{th} week, whereas, during 10^{th} to 12^{th} week, it was increased from 86.9 to 96.0%. Similarly, RH_{eve} was increased from 60.9 to 67.1% during 7^{th} to 8^{th} week and 67.1 to 81.0% from 10^{th} to 12^{th} week. Rainfall was 65mm and 30.5mm during 7^{th} and 8^{th} week. Whereas, during 10^{th}, 11^{th} and 12^{th} week it was 69.0, 76.5 and 154.2mm (Annexure-I).

The perusal of data from Table 4.8 revealed that white fly population showed very high fluctuation during 2014. After an increase in population to 116.4 and 121.7 whitefly during 10^{th} and 11^{th} week in HS-6 and RCH 134 BG-II, respectively, the population started to decrease up to 13^{th} week in both the cultivars. Thereafter, sudden increase in number in the population was observed during 15^{th} week in both the cultivars. The white fly population was 276.3 on HS-6 and 293.7 on RCH 134 BG-II. The weather variable during the period of fluctuation showed that the T_{max} was increased from 35.2 to 38.5 ^{o}C at 11^{th} to 13^{th} week and decreased to 33.0 ^{o}C at 15^{th} week. Similarly, T_{min} showed little change of 26.9 to 25.8 ^{o}C during 11^{th} to 13^{th} week and 25 ^{o}C at 15^{th} week. Data revealed that RH_{mor} was decreased from 91.0 to 70% during 11^{th} to 13^{th} week after sowing and then increased again up to 92% at 15^{th} week after sowing. Similarly, RH_{eve} also decreased from 64.0 to 37.0% during 11^{th} to 13^{th} week and again increased to 67.0% at 15^{th} week after sowing. After a rainfall of 33.0mm on 10^{th} week there was dry spell up to 13^{th} week. Rainfall was observed on 14^{th} week (1.0mm) and remained continued in 15^{th} week (76.7mm) during 2014 (Annexure-II).

4.2.1.3.7 Seed cotton yield

Incidence of CLCuD on cotton also affected the plant yield performance. The incidence and disease severity significantly affected the plant yield. Perusal of data in Table 4.9 revealed the seed cotton yield (SCY) of plants of different CLCuD grades. The early incidence of CLCuD was observed during 2013 and plants started to show disease symptoms at early stage. Therefore, at the harvesting time the plants under disease grade 2 and 3 were not available for yield comparison. Data presented in Table 4.9 revealed that plants showing disease grade 4, 5 and 6 produced SCY 1105.4, 783.9 and 518.6 Kg/ha, respectively

in HS-6. Similarly, in cv. RCH 134 BG-II, SCY of disease grade 4, 5 and 6 plants was 1273.4, 901.8 and 694.4 Kg/ha, respectively during 2013 (Table 4.9). The high R^2 value indicated that the contribution of CLCuD in the reduction of SCY was up to 81% during 2013.

Table 4.9 Seed cotton yield of plants of different disease grades

CLCuD grade	Seed cotton yield (Kg/ha)	
	2013	**2014**
	HS-6	
2	-	1353.0
3	-	1244.3
4	1105.4	1194.4
5	783.9	867.1
6	518.6	614.3
CD (P= 0.05)	**64.5**	**87.3**
CV (%)	**16.8**	**18.2**
	RCH 134 BG-II	
2	-	1413.3
3	-	1315.0
4	1273.4	1287.4
5	901.8	1016.2
6	694.4	806.8
CD (P= 0.05)	**92.3**	**76.7**
CV (%)	**21.4**	**24.4**

Whereas, data in table revealed that plants categorized under disease grade 2 to 6 were available at the time of crop harvest during 2014. Seed cotton yield was ranged from 614.3 to 1353.0 Kg/ha in HS-6 and 806.8 to 1413.3 Kg/ha in RCH 134 BG-II. Yield obtained in different graded plants showed significant differences according to the disease severity. Seed cotton yield showed reciprocal relationship with the disease grade. Plants with lower disease grade showed higher SCY as compared to the plants with higher disease grade (Table 4.9). The data was subject to regression analysis and high R^2 value indicated that

contribution of CLCuD in the reduction of SCY was up to 82% during 2014. Among the two cultivars, RCH 134 BG-II gave more SCY as compared to HS-6 for all the disease grades (Table 4.9).

4.2.2 Effect of date of sowing and plant spacing

4.2.2.1 Disease appearance and disease severity

Effect of date of sowing and plant spacing on the incidence of CLCuD was studied on the two cotton cultivars HS-6 and RCH 134 BG-II. The crop was sown on three different date: early (1st May), normal (15th May) and late (1st June) along with three plant spacing *viz.* 67.5 x 60, 67.5 x 30 and 67.5 x 15cm^2 for each date. The perusal of data in Table 4.10 revealed that appearance of disease on cotton cv. HS-6 and RCH 134 BG-II in early sown crop was observed at 43 and 41 DAS, respectively, whereas, it was at 30 and 29 DAS on normal sowing date and at 13 and 12 DAS on late sown crop, respectively. Disease intensity was also calculated and data presented in Table 4.10 revealed that maximum disease intensity was observed in late sown crop at 15cm spacing on HS-6 (65.28%) and 64.89% on RCH 134 BG-II. The least disease intensity 14.60% and 31.78% was observed on HS-6 and RCH 134 BG-II, respectively in early sown crop at spacing 60cm. The effect of spacing on disease intensity was significant on HS-6 and maximum disease intensity was observed at 15cm, followed by 30cm and 60cm spacing for all the sowing date, whereas, effect to spacing on disease intensity was non significant on RCH 134 BG-II during 2013 (4.10).

Table 4.10 Effect of date of sowing and plant spacing on disease appearance and severity of CLCuD on two cotton cultivars during 2013

Date of sowing	Disease appearance (DAS)		HS-6				RCH 134 BG-II			
			Disease severity (%)							
	HS-6	RCH 134 BG-II	15 cm	30 Cm	60 cm	Mean	15 cm	30 cm	60 cm	Mean
1.5.2013	43	41	27.54	18.77	14.6	**20.3**	37.94	33.85	31.78	**34.5**
15.5.2013	30	29	47.17	39.76	32.46	**39.8**	48.17	43.29	46.31	**45.9**
1.6.2013	13	12	65.28	62.42	59.44	**62.4**	64.89	63.41	59.84	**62.7**
Mean			**46.7**	**40.3**	**35.5**		**50.3**	**46.9**	**46**	
CD at 5%										
DOS			**1.75**				**1.84**			
Spacing			**1.56**				**NS**			
DOS x Spacing			**2.34**				**NS**			

Similarly, Table 4.11 revealed that the disease first appeared at 44 and 45 DAS on HS-6 and RCH 134 BG-II, respectively in the early sown crop, whereas, in normal sown crop, disease appeared at 37 DAS in both the cultivars during 2014. In late sown crop, the disease appeared at early crop stage i.e. 28 and 27

DAS on HS-6 and RCH 134 BG-II, respectively. Perusal of data from Table 4.11 revealed that the maximum disease intensity was observed on late sown crop at 15cm spacing on HS-6 (38.17%) and 29.88 on RCH 134 BG-II in 2014. Whereas, least disease intensity 18.77% and 21.83% was observed in the early sown crop at spacing 60cm on HS-6 and RCH 134 BG-II, respectively. Data presented in Table 4.11 revealed that there was significant effect of spacing on disease intensity on cv. HS-6. Maximum disease intensity was observed at 15cm, followed by 30cm and 60cm spacing for all the sowing date on HS-6 during 2014, whereas, effect of spacing on disease intensity was observed non significant on RCH 134 BG-II during 2014.

Table 4.11 Effect of date of sowing and plant spacing on disease appearance and severity of CLCuD on two cotton cultivars during 2014

Date of sowing	Disease appearance (DAS)		HS-6				RCH 134 BG-II			
			Disease severity (%)							
	HS-6	RCH 134 BG-II	15 cm	30 cm	60 cm	Mean	15 cm	30 cm	60 cm	Mean
1.5.2014	44	45	22.58	19.92	18.77	**20.4**	23.29	23.81	21.83	**23.0**
15.5.2014	37	37	30.28	30.50	30.09	**30.3**	28.06	29.02	28.35	**28.5**
1.6.2014	28	27	38.17	37.70	37.75	**37.9**	29.88	29.63	29.55	**29.7**
Mean			**30.3**	**29.4**	**28.9**		**27.1**	**27.5**	**26.6**	
CD at 5%										
DOS			**0.99**				**3.15**			
Spacing			**0.88**				**NS**			
DOS x Spacing			**NS**				**NS**			

4.2.2.2 Area under disease progress curve

Area under disease progression was calculated as per the formula of Wilcoxon *et al*. (1975) for three date of sowing and three plant spacing. The perusal of data in Table 4.12 revealed that AUDPC was ranged from 0.67 to 230.43 for 15cm spacing, 1.96 to 241.20 for 30cm spacing and 1.75 to 334.85 for 60cm spacing in early sown crop. Whereas, AUDPC of normal sown crop was range from 0.85 to 274.67 for 15cm, 1.52 to 336.91 for 30cm and 6.75 to 396.57 for 60cm spacing, respectively. Under late sown crop, AUDPC was ranged from 5.85 to 385.77, 4.38 to 367.25 and 5.66 to 383.20 for 15cm, 30cm and 60cm spacing, respectively in cv. HS-6 during 2013.

Similarly, the perusal of data in Table 4.13 revealed that the AUDPC was ranged from 11.67 to 367.49 for 15cm spacing, 8.38 to 316.93 for 30cm spacing and 7.78 to 293.60 for 60cm spacing in early sown crop. Whereas, AUDPC of normal sown crop was range from 30.92 to 449.15 for 15cm, 25.90 to 386.93 for

30cm and 17.62 to 429.71 for 60cm spacing. For late sown crop AUDPC was ranged from 18.59 to 569.70, 22.36 to 567.76 and 9.31 to 505.54 for 15cm, 30cm and 60cm spacing, respectively in cv RCH 134 BG-II during 2013. The data revealed that delay in time of sowing fetch more amount of disease. However, effect of spacing was not observed in the disease severity for any date of sowing in both the cultivars.

The perusal of data in Table 4.14 revealed that AUDPC was ranged from 11.0 to 536.0 for 15cm spacing, 8.6 to 266.7 for 30cm spacing and 6.5 to 256.4 for 60cm spacing in early sown crop. Whereas, AUDPC of normal sown crop was range from 8.3 to 289.4 for 15cm, 4.6 to 573.6 for 30cm and 3.7 to 485.7 for 60cm spacing. For late sown crop AUDPC was ranged from 7.4 to 333.6, 6.5 to 449.8 and 5.8 to 511.4 for 15cm, 30cm and 60cm spacing, respectively on cv. HS-6 during 2014.

Similarly, data from Table 4.15 revealed that AUDPC on cv. RCH 134 BG-II was varied from 4.40 to 289.62 for 15cm spacing, 5.74 to 275.03 for 30cm spacing and 3.83 to 316.10 for 60cm spacing in early sown crop. Whereas, AUDPC of normal sown crop was range from 3.71 to 367.25 for 15cm, 0.93 to 371.50 for 30cm and 1.11 to 459.50 for 60cm spacing. For late sown crop AUDPC was ranged from 11.23 to 395.82, 9.72 to 481.61 and 11.11 to 439.31 for 15cm, 30cm and 60cm spacing, respectively in cv RCH 134 BG-II during 2014. Data from the tables revealed that, AUDPC of late sown crop was highest, whereas, early sown crop exhibited lowest value for all sowing date.

Table 4.12 Area under disease progress curve for CLCuD on cv. HS-6 sown at different date of sowing and plant spacing during 2013

St. Met. Weeks	DOS 1.5.2013			DOS 15.5.2013			DOS 1.6.2013		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
29	-	-	-	-	-	-	15.8	13.0	10.2
30	-	-	-	0.9	1.7	9.4	25.7	18.4	14.5
31	17.5	5.8	1.9	24.8	22.0	10.7	85.0	73.7	55.2
32	60.3	17.5	5.8	42.4	36.9	19.4	151.7	145.8	116.7
33	106.9	36.9	11.7	73.5	62.2	38.9	180.2	176.0	153.6
34	140.0	58.3	21.4	130.3	106.9	66.1	230.8	201.3	178.9
35	163.3	81.7	38.9	186.7	147.8	101.1	278.0	231.4	202.7
36	186.7	116.7	62.2	215.8	173.0	143.9	301.3	270.3	228.0
37	221.7	147.8	85.6	268.3	221.7	188.6	340.3	305.3	264.4
38	260.5	175.0	128.3	332.5	270.3	223.6	383.0	346.1	320.8
39	291.7	210.0	173.0	382.6	338.3	260.5	423.9	402.5	375.3
40	318.9	243.0	208.0	433.7	398.6	305.3	464.7	443.3	414.2
41	348.0	274.2	248.9	481.6	421.9	346.1	499.7	486.1	462.8

Table 4.13 Area under disease progress curve for CLCuD on cv. RCH 134 BG-II sown at different date of sowing and plant spacing during 2013

St. Met. Weeks	DOS 1.5.2013			DOS 15.5.2013			DOS 1.6.2013		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
28	-	-	-	-	-	-	18.6	22.4	9.3
29	-	-	-	30.9	25.9	17.6	55.2	44.1	50.1
30	11.7	8.4	5.6	40.6	38.9	31.1	118.5	95.4	93.3
31	27.2	25.3	13.4	68.1	56.4	42.8	169.2	140.0	122.5
32	44.7	31.2	17.6	93.3	97.2	58.3	202.2	186.7	151.7
33	64.2	40.8	23.1	112.8	120.6	87.5	231.4	221.7	194.4
34	93.3	60.3	32.7	141.9	134.2	128.3	256.7	245.0	223.6
35	128.3	89.4	56.4	163.3	153.6	151.7	291.7	270.3	246.9
36	169.2	116.7	95.3	192.5	171.1	173.1	316.9	297.5	278.0
37	221.7	153.6	136.1	243.1	202.2	221.7	346.1	326.7	322.8
38	268.3	198.3	176.9	295.5	245.0	276.1	406.4	361.7	371.4
39	299.4	237.2	219.7	338.3	295.5	330.5	466.7	408.3	396.7
40	328.6	278.0	266.4	392.8	340.3	381.1	515.3	478.3	441.4
41	367.5	316.9	293.6	449.2	386.9	429.7	569.7	567.8	505.5

Table 4.14 Area under disease progress curve for CLCuD on cv. HS-6 sown at different date of sowing and plant spacing during 2014

St. Met. Weeks	DOS 1.5.2014			DOS 15.5.2014			DOS 1.6.2014		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
30	-	-	-	-	-	-	11.7	13.6	10.5
31	-	-	-	9.7	11.7	7.8	46.7	44.7	43.2
32	15.6	13.6	11.7	36.9	40.8	35.0	108.9	104.2	106.0
33	56.4	44.7	38.9	77.8	77.8	79.7	167.2	162.6	163.5
34	106.9	71.9	64.2	118.6	117.8	125.8	202.2	196.8	194.6
35	141.9	97.2	85.6	155.6	152.8	158.7	231.4	232.0	233.1
36	159.4	126.4	112.8	194.4	186.7	187.4	268.3	267.8	272.6
37	180.8	157.5	141.9	233.3	231.4	230.2	311.1	303.9	308.2
38	210.0	184.7	171.1	278.1	274.2	271.8	350.0	336.0	350.2
39	245.0	213.9	206.1	328.6	334.4	323.0	390.8	378.6	393.2
40	280.0	256.7	243.0	383.0	400.5	387.3	429.7	429.3	423.5
41	307.2	293.6	281.9	435.5	439.4	433.6	464.7	465.1	449.9

Table 4.15 Area under disease progress curve for CLCuD on cv. RCH 134 BG-II sown at different date of sowing and plant spacing during 2014

St. Met. Weeks	DOS 1.5.2014			DOS 15.5.2014			DOS 1.6.2014		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
29	-	-	-	-	-	-	3.9	5.8	4.7
30	-	-	-	13.6	17.5	15.6	15.6	25.3	20.2
31	11.7	12.1	7.8	36.9	56.4	46.7	42.8	54.4	48.8
32	38.9	41.2	27.2	68.1	97.2	81.7	83.6	85.6	82.2
33	70.0	81.7	56.4	108.9	128.3	121.5	126.4	116.7	122.5
34	106.9	118.6	95.3	147.8	154.2	154.0	165.3	154.4	164.1
35	153.6	159.4	136.1	186.7	181.8	181.4	196.4	190.4	193.7
36	196.4	204.2	176.9	227.5	214.5	219.7	233.3	226.5	228.7
37	231.4	233.3	219.7	272.2	267.2	272.4	276.1	267.9	267.6
38	270.3	266.4	266.4	311.1	326.3	315.4	316.9	309.5	305.7
39	308.2	307.2	293.6	342.2	364.0	356.0	365.5	364.4	357.0
40	343.2	346.3	317.9	379.1	386.9	381.5	418.0	416.1	411.8
41	366.2	372.7	356.1	425.8	416.5	406.6	455.0	455.0	457.5

4.2.2.3 Apparent infection rate

The apparent infection rate of CLCuD on both the cultivars was calculated for three date of sowing and plant spacing by using the formula given by Vanderplank (1963). The perusal of data in Table 4.16 revealed that apparent infection rate was varied from 0.06 to 0.47 at 15cm spacing, 0.10 to 0.53 at 30cm and 0.06 to 0.51 at 60cm spacing in the early sown crop on cv. HS-6 during 2013. Apparent infection rate of disease on plants sown on normal date was ranged from 0.01 to 0.49 for 15cm, 0.05 to 0.51 for 30cm and 0.01 to 53 for 60cm spacing. Range of AUDPC was 0.03 to 0.53 for 15cm, 0.02 to 0.53 for 30cm and 0.04 to 0.53 for 60cm spacing under late sown condition. The apparent infection rate under late sown condition was not much different in plants for three spacing.

Similarly, the perusal of data in Table 4.17 revealed that the AIR was varied from 0.11 to 0.48 for 15cm spacing, 0.11 to 0.46 for 30cm spacing and 0.03 to 0.46 for 60cm spacing in early sown crop. Whereas, AIR of normal sown crop was range from 0.11 to 0.51 for 15cm, 0.06 to 0.49 for 30cm and 0.01 to 0.50 for 60cm spacing. For late sown crop AIR was ranged from 0.13 to 0.54, 0.04 to 0.53 and 0.16 to 0.52 for 15cm, 30cm and 60cm spacing, respectively in cv RCH 134 BG-II during 2013.

The perusal of data in Table 4.18 revealed that apparent infection rate was varied from 0.16 to 0.47 at 15cm spacing, 0.14 to 0.47 at 30cm and 0.12 to 0.46 at 60cm spacing in the early sown crop on cv.

HS-6 during 2014. AIR of disease on plants sown on normal date was ranged from 0.09 to 0.52 for 15cm, 0.12 to 0.52 for 30cm and 0.05 to 52 for 60cm spacing. Range of AIR was 0.12 to 0.52 for 15cm, 0.14 to 0.52 for 30cm and 0.10 to 0.52 for 60cm spacing under late sown condition. Data from the table revealed that the plant spacing not showed much variations in the infection rate during 2014.

Similarly, the perusal of data in Table 4.19 revealed that the AIR on RCH 134 BG-II during 2014 was varied from 0.12 to 0.49 for 15cm spacing, 0.12 to 0.49 for 30cm spacing and 0.05 to 0.48 for 60cm spacing in early sown crop. Whereas, AIR of normal sown crop was range from 0.14 to 0.51 for 15cm, 0.18 to 0.51 for 30cm and 0.16 to 0.24 for 60cm spacing. For late sown crop AIR was ranged from 0.15 to 0.50, 0.01 to 0.50 and 0.07 to 0.49 for 15cm, 30cm and 60cm spacing, respectively during 2014. Data revealed that plant spacing had not much effect on the infection rate whereas, time of sowing showed effect on the infection rate. The experimental data revealed that AIR was highest in late sown condition whereas, early sown crop exhibited lowest AIR value.

Table 4.16 Apparent infection rate for CLCuD on cv. HS-6 sown at different date of sowing and plant spacing during 2013

St. Met. Weeks	DOS 1.5.2013			DOS 15.5.2013			DOS 1.6.2013		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
29	-	-	-	-	-	-	0.03	0.14	0.04
30	-	-	-	0.07	-	-	0.02	0.02	0.03
31	0.10	0.06	0.05	0.18	0.05	0.09	0.12	0.07	0.13
32	0.14	0.14	0.12	0.36	0.11	0.01	0.35	0.31	0.34
33	0.25	0.22	0.19	0.39	0.21	0.14	0.39	0.37	0.38
34	0.31	0.29	0.26	0.41	0.28	0.23	0.40	0.39	0.40
35	0.39	0.36	0.3	0.45	0.36	0.3	0.42	0.41	0.43
36	0.41	0.38	0.33	0.46	0.38	0.35	0.44	0.42	0.44
37	0.43	0.40	0.36	0.47	0.41	0.39	0.46	0.44	0.46
38	0.47	0.44	0.39	0.49	0.44	0.42	0.47	0.46	0.47
39	0.49	0.46	0.42	0.50	0.46	0.43	0.49	0.48	0.49
40	0.50	0.50	0.44	0.51	0.49	0.46	0.51	0.50	0.51
41	0.52	0.50	0.45	0.52	0.50	0.47	0.52	0.51	0.52

Table 4.17 Apparent infection rate for CLCuD on cv. RCH 134 BG-II sown at different date of sowing and plant spacing during 2013

St. Met. Weeks	DOS 1.5.2013			DOS 15.5.2013			DOS 1.6.2013		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
30	-	-	-	0.11	0.06	0.01	0.13	0.04	0.16
31	-	-	-	0.19	0.17	0.11	0.30	0.28	0.28
32	0.11	0.11	0.04	0.21	0.21	0.19	0.38	0.34	0.34
33	0.16	0.14	0.03	0.31	0.28	0.22	0.40	0.39	0.35
34	0.25	0.18	0.09	0.31	0.35	0.28	0.42	0.42	0.40
35	0.37	0.33	0.28	0.39	0.38	0.38	0.47	0.46	0.45
36	0.41	0.35	0.34	0.42	0.40	0.41	0.47	0.47	0.46
37	0.44	0.40	0.38	0.45	0.42	0.44	0.49	0.48	0.48
38	0.46	0.42	0.41	0.47	0.45	0.46	0.51	0.49	0.50
39	0.47	0.44	0.44	0.49	0.47	0.49	0.53	0.51	0.50
40	0.48	0.46	0.46	0.51	0.49	0.50	0.54	0.53	0.52

Table 4.18 Apparent infection rate for CLCuD on cv. HS-6 sown at different date of sowing and plant spacing during 2014

St. Met. Weeks	DOS 1.5.2014			DOS 15.5.2014			DOS 1.6.2014		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
29	-	-	-	-	-	-	0.12	0.14	0.10
30	-	-	-	0.09	0.12	0.05	0.28	0.26	0.27
31	0.16	0.14	0.12	0.24	0.25	0.24	0.37	0.37	0.37
32	0.30	0.26	0.24	0.32	0.32	0.33	0.40	0.40	0.40
33	0.36	0.30	0.28	0.36	0.36	0.37	0.42	0.42	0.42
34	0.38	0.34	0.32	0.39	0.39	0.39	0.44	0.44	0.44
35	0.39	0.37	0.35	0.42	0.42	0.41	0.46	0.46	0.46
36	0.41	0.39	0.38	0.44	0.44	0.44	0.48	0.47	0.48
37	0.43	0.41	0.40	0.46	0.46	0.46	0.49	0.48	0.49
38	0.45	0.43	0.43	0.48	0.49	0.49	0.50	0.50	0.50
39	0.46	0.45	0.45	0.50	0.51	0.50	0.51	0.52	0.51
40	0.47	0.47	0.46	0.52	0.52	0.52	0.52	0.52	0.52
41	0.20	0.19	0.19	0.24	0.24	0.24	0.25	0.25	0.25

Table 4.19 Apparent infection rate for CLCuD on cv. RCH 134 BG-II sown at different date of sowing and plant spacing during 2014

St. Met. Weeks	DOS 1.5.2014			DOS 15.5.2014			DOS 1.6.2014		
	15cm	30cm	60cm	15cm	30cm	60cm	15cm	30cm	60cm
29	-	-	-	-	-	-	0.15	0.01	0.07
30	-	-	-	0.14	0.18	0.16	0.12	0.19	0.16
31	0.12	0.12	0.05	0.26	0.27	0.27	0.22	0.29	0.26
32	0.24	0.25	0.19	0.33	0.32	0.32	0.31	0.34	0.32
33	0.30	0.33	0.28	0.37	0.36	0.37	0.35	0.37	0.37
34	0.35	0.36	0.34	0.40	0.40	0.40	0.39	0.39	0.39
35	0.40	0.40	0.38	0.42	0.41	0.42	0.42	0.41	0.41
36	0.42	0.42	0.41	0.44	0.44	0.44	0.44	0.43	0.44
37	0.44	0.44	0.44	0.46	0.46	0.46	0.46	0.46	0.46
38	0.46	0.46	0.46	0.48	0.48	0.47	0.47	0.48	0.48
39	0.47	0.47	0.46	0.50	0.50	0.50	0.49	0.49	0.49
40	0.49	0.49	0.48	0.51	0.51	0.51	0.50	0.50	0.49
41	0.20	0.21	0.20	0.23	0.24	0.24	0.23	0.22	0.22

4.2.2.4 Agronomic traits

Data on various agronomic traits on twenty selected (tagged) plants of cotton for each date of sowing and spacing was observed. Effect of incidence of CLCuD and its effect on various agronomic and yield attributing traits of plants were studied. The characters included for the study *viz.* plant height, boll number, boll weight, seed cotton yield, lint index and seed index.

4.2.2.4.1 Plant Height

Perusal of data in Table 4.20 exhibited that maximum average plant height of HS-6 in early sown (155.9 cm) and timely sown (147.8 cm) were non-significant. Whereas, late sown crop showed significant reduction in plant height (120.2 cm) as compared to early and timely sown crops. Similarly, maximum average height of plants in RCH 134 BG-II was 156.0cm and 149.0cm in early and timely sown crops, respectively, which were significantly different from average plant height (120.4 cm) of late sown crop.

The data in Table 4.21 revealed that maximum plant height of HS-6 (156.0cm) in early sown crop was not significantly different from timely sown crop (153.1cm). Whereas, plant height 121.9cm of late sown crop was significantly lower than the early and timely sown crop. Similarly, in RCH 134 BG-II, plant height in late sown crop (131.1 cm) was significantly lower than that of early (159.3) and timely sown (154.3 cm) crop.

4.2.2.4.2 Boll number

Perusal of data in Table 4.20 exhibited that the average boll number of early and timely sown crop was 27.2 and 20.4 per plant respectively and were significantly higher than that of late sown crop (10.8 bolls) on HS-6 during 2013. Similarly, data revealed that on RCH 134 BG-II, boll number was 28.9, 19.4 and 13.0 in early, timely and late sown crops respectively. Boll number of late sown crop (13.0) was significantly lower that the early and timely sown crop.

Data from Table 4.21 on boll number during 2014 revealed that there was significant higher number of bolls on HS-6 from early (36.4) and timely (33.7) sown crop as compared to the late sown (26.6) crop. Similarly, on RCH 134 BG-II early and timely (38.2 and 35.8) sown crop showed significant increase in boll number over the late sown crop with average boll number 28.5.

4.2.2.4.3 Boll weight

Perusal of data from Table 4.20 exhibited that the boll weight of HS-6 cultivar was 3.7g and 3.2g in early and timely sown crop, respectively and were not significantly different from each other. Whereas, boll weight of late sown crop (2.6g) was significantly lower than the early and timely sown crop. Similarly, data also revealed that on RCH 134 BG-II, average boll weight of early (3.8g) and timely (3.4g) sown crop was not significantly different from each other, whereas, boll weight of late sown crop (2.4g) was significantly low during 2013. Data detailed in Table 4.21 revealed that boll weight 3.8g of early and 3.5g of timely sown crop of HS-6 were not significantly different, whereas, boll weight 2.5g of late sown crop was significantly different from early and timely sown crop of HS-6. Data also exhibited that boll weight of RCH 134 BG-II for early and timely sown (4.0g and 3.7g, respectively) was non significant during 2014. However, late sown crop showed significantly reduced boll weight (2.8g) as compared to the early and timely sown crop.

4.2.2.4.4 Seed cotton yield (SCY)

Data on SCY mentioned in Table 4.20 for the seasons 2013 revealed that SCY was significantly highest in early (687.7 Kg/ha) and timely sown crop (621.7 Kg/ha) as compared to the late sown crop which showed significantly lowest average yield 428.7 Kg on HS-6. Similarly, average SCY of RCH 134 BG-II from early (718.7Kg/ha) and timely sown crop (645.3Kg/ha) was significantly higher than 491.0Kg/ha yield of late sown crop.

Perusal of data in Table 4.21 revealed that highest SCY 842.7Kg/ha observed in early sown crop was not significantly different from timely sown crop (771.3 Kg/ha). Whereas, SCY 614.7Kg/ha from the late sown crop of HS-6 was significantly lowest (Table 4.21).

4.2.2.4.5 Ginning out turn

The data from Table 4.20 revealed that during 2013 ginning out turn (GOT) of HS-6 was 42.3% for early sown crop followed by 36.7 % of timely sown crop. GOT of late sown crop (25.5%) was significantly lower than the early and timely sown crops. Similarly, GOT of RCH 134 BG-II for early and timely sown crop was 40.7% and 37.5%, respectively and was significantly higher than of late sown crop (27.1%).

Perusal of data in Table 4.21 exhibited that GOT of HS-6 of early (36.5%) and timely (34.2) sown crop was significantly higher than that of late sown crop (27.9%). Highest, GOT of RCH 134 BG-II was from early sown crop (36.3%) followed by timely sown crop (26.3%). GOT of late sown crop was 13.1%, which was significantly lower than the GOT of early and timely sown cotton crops.

4.2.2.4.6 Seed and lint index

Perusal of data from Table 4.20 exhibited that the seed index of HS-6 for early and timely sown crop was 7.8 and 7.4, respectively during 2013. Whereas, seed index 4.7 of late sown crop was significantly different low of cv. HS-6. Similarly, seed index 8.6 and 8.1 for early and timely sown crop, respectively was not significantly different from each other for RCH 134 BG-II. Whereas, seed index 5.6 of late sown crop was significantly lower from early and timely sown crops on RCH 134 BG-II. Similarly, perusal of data in Table 4.21 revealed that seed index for early and timely sown crop of HS-6 (9.0 and 8.7, respectively) was significantly higher than the late sown crop (6.3). Seed index of RCH 134 BG-II for early and timely sown (8.5 and 7.0, respectively) was also significantly higher than the late sown crops with (3.9).

Data from Table 4.20 revealed that lint index also followed similar trend as of the other agronomic parameters. Data exhibited that during 2013 highest lint index 5.7 of early sown crop closely followed by timely sown crop (3.8) were non significant to each other on HS-6. Whereas, lowest lint index 1.6 of HS-6 in late sown condition was significantly low from early and timely sown crops. Similarly, in RCH 134 BG-II, lint index 5.9 and 4.8 for early and timely sown crop respectively was significantly higher than the lint index (2.1) of late sown crop during 2013. Whereas, data presented in Table 4.21 for lint index revealed that highest lint index of HS-6 from early sown crop (5.1) was significantly higher than that from timely and late sown crop with 4.5 and 2.4 lint index respectively. Whereas, lint index 5.6 of RCH 134 BG-II from early sown crop followed by timely (4.2) and late sown crops (2.8) were significantly different from each other.

4.2.4 Effect of spacing on agronomic traits

Effect of plant spacing was studied for its effect on incidence and severity of CLCuD. Effect of diseased plants on various plant characters was observed.

Perusal of data in Table 4.22 revealed that effect of plant spacing on boll number was non significant on HS-6 at spacing 15cm (16.8) and 30cm (17.6) during 2013. Whereas, at spacing 60cm plants showed significantly high number of bolls (24.1) during 2013. Spacing had no effect on boll number in HS-6 however, maximum SCY was observed at 15cm (727.3Kg/ha) followed by 30cm (549.3Kg/ha) and 60cm (461.3Kg/ha). Lint index was highest at 60cm (4.0) spacing followed by 15cm (3.6) and 30cm (3.4). Whereas, effect of spacing on other characters was non significant.

Similarly, data from Table 4.22 also exhibited that boll number in RCH 134 BG-II was 18.4 at 15cm spacing followed by 18.7 at 30cm spacing were non significant. Whereas, significantly high increase in number was observed at 60cm spacing (24.3bolls). Similarly, data also revealed that boll weight was significantly highest from plants sown at 60cm spacing as compared to the 15cm and 30cm spacing in the cv. RCH 134 BG-II during 2013. SCY of plants at closer spacing of 15cm showed maximum SCY (757.7Kg/ha) followed by 588.0 Kg/ha in 30cm and 509.3Kg/ha in 60cm spacing for RCH 134 BG-II during 2013. Whereas, effect of spacing on other characters was non significant during 2013.

Data presented in Table 4.23 revealed that the plant spacing had significant effect on number of bolls per plant during 2014. Data exhibited that at 15cm spacing, numbers of bolls per plant were 28.0, followed by 32.2 bolls at 30cm and maximum 36.6 bolls per plant were observed at 60cm spacing on HS-6 during 2014. SCY was significantly highest (854.3Kg/ha) at 15cm spacing, followed by 30cm (670.7Kg/ha) and the lowest yield 403.7Kg/ha was obtained from at 60cm spacing. Similarly, data form table also revealed that boll number on plants at 15cm significantly reduced to minimum 29.0 bolls on RCH 134 BG-II. Whereas, maximum bolls 39.0 were observed at 60cm spacing on RCH 134 BG-II during 2014. Perusal of data in Table 4.23 revealed that SCY at 15cm spacing was maximum 945.3KG/ha, followed by 30cm (695.3Kg/ha) and 60cm (386.3Kg/ha) spacing on cv. RCH 134 BG-II. Data presented in Table 4.23 revealed that the GOT of RCH 134 BG-II at 15cm and 30cm distance was not significantly different (23.8% and 24.8% respectively). Whereas, at 60cm spacing it was significantly highest (27.2%). Data presented in Table 4.23 also revealed that lint index at 15cm spacing was significantly lowest (3.3) as compared to 30cm (3.8) and 60cm (4.1). Effect of spacing on other characters was non significant during 2014.

Perusal of data from table 4.20 and 4.21 revealed that the time of sowing can significantly affect the plant characters that have affect the response of plant towards the disease incidence on plant. Whereas, table 4.22 and 4.23 revealed that plant spacing comparatively had low effect on plant characters. However, significantly effect on number of bolls per plant, SCY and lint index was observed.

Table 4.20 Effect of time of sowing on disease severity and on different yield attributes in cotton during 2013

Date of sowing	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg)/ha	GOT (%)	Seed index	Lint index	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg)/ha	GOT (%)	Seed index	Lint index
	HS-6								RCH 134 BG-II							
1.5.13	20.3	155.9	27.2	3.7	687.7	42.3	7.8	5.7	34.5	156.0	28.9	3.8	718.7	40.7	8.6	5.9
15.5.13	39.8	147.8	20.4	3.2	621.7	36.7	7.4	3.8	45.9	149.0	19.4	3.4	645.3	37.5	8.1	4.8
1.6.13	62.4	120.2	10.8	2.6	428.7	25.5	4.7	1.6	62.7	120.4	13.0	2.4	491.0	27.1	5.6	2.1
CD (at 5%)	**10.98**	**13.0**	**3.6**	**0.5**	**28.4**	**7.9**	**1.5**	**2.0**	**8.2**	**12.7**	**1.0**	**0.8**	**59.9**	**8.6**	**2.4**	**2.3**

PH = plant height, SCY = seed cotton yield and GOT = ginning out turn

Table 4.21 Effect of time of sowing on disease severity and on different yield attributes in cotton during 2014

Date of Sowing	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg) /ha	GOT (%)	Seed index	Lint index	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg) /ha	GOT (%)	Seed index	Lint index
	HS-6								RCH 134 BG-II							
1.5.13	20.42	156.0	36.4	3.8	842.7	36.5	9.0	5.1	22.97	159.3	38.2	4.0	892.7	36.3	8.5	5.6
15.5.13	26.29	153.1	33.7	3.6	771.3	34.2	8.7	4.5	28.48	154.3	35.8	3.7	781.3	28.3	7.0	4.2
1.6.13	37.87	121.9	26.6	2.5	614.7	27.9	6.3	2.4	39.65	131.1	28.5	2.8	653.0	13.1	3.9	2.8
CD (at 5%)	**8.20**	**23.1**	**7.9**	**0.9**	**39.1**	**5.4**	**2.2**	**1.7**	**6.80**	**1.6**	**1.3**	**0.7**	**24.4**	**9.7**	**2.2**	**1.2**

PH = plant height, SCY = seed cotton yield and GOT = ginning out turn

Table 4.22 Effect of plant spacing on disease severity and on different yield attributes in cotton during 2013

Spacing	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg)/ha	GOT (%)	Seed index	Lint index	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg)/ha	GOT (%)	Seed index	Lint index
	HS-6								RCH 134 BG-II							
15cm	46.7	141.4	16.8	3.1	727.3	34.1	6.5	3.6	50.3	142.0	18.4	3.1	757.7	36.1	7.4	4.4
30cm	40.3	141.4	17.6	3.1	549.3	32.4	6.6	3.4	46.9	142.4	18.7	3.2	588.0	35.1	7.5	4.3
60cm	35.5	141.1	24.1	3.2	461.3	35.0	6.7	4.0	46.0	141.0	24.3	3.4	509.3	34.1	7.4	4.2
CD (at 5%)	**NS**	**NS**	**4.7**	**NS**	**28.4**	**NS**	**NS**	**0.4**	**NS**	**NS**	**5.8**	**0.1**	**26.5**	**NS**	**NS**	**NS**

PH = plant height, SCY = seed cotton yield and GOT = ginning out turn

Table 4.23 Effect of plant spacing on disease severity and on different yield attributes in cotton during 2014

Spacing	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg) /ha	GOT (%)	Seed index	Lint index	Disease severity (%)	PH (cm)	Boll numbers	Boll weight (g)	SCY (Kg) /ha	GOT (%)	Seed index	Lint index
	HS-6								RCH 134 BG-II							
15cm	30.34	144.0	28.0	2.8	854.3	33.5	8.0	4.1	27.07	148.9	29.0	3.2	945.3	23.8	6.5	3.3
30cm	29.37	143.7	32.2	3.6	670.7	32.7	8.0	4.1	27.45	147.7	34.5	3.7	695.3	24.8	6.4	3.8
60cm	28.87	143.3	36.6	4.1	403.7	32.4	7.9	3.9	26.57	148.0	39.0	4.0	386.3	27.2	6.4	4.1
CD (at 5%)	**NS**	**NS**	**2.9**	**NS**	**39.1**	**NS**	**NS**	**0.2**	**NS**	**NS**	**4.4**	**NS**	**78.3**	**3.1**	**NS**	**0.2**

PH = plant height, SCY = seed cotton yield and GOT = ginning out turn

4.3 Biochemical Studies

The aim of the study was to analyse the biochemical constituents of cotton cultivars which showed susceptible and resistant reactions to CLCuD. During the early stages of crop (up to 30DAS), susceptible plants achieved the symptoms up to disease grade-2. Therefore, the biochemical tests with higher disease grade were done only after 30DAS. The results for various biochemical tests are detailed under the different heads.

4.3.1 Phenol

Perusal of data from Table 4.24 and Fig 4.5 revealed that phenol content was increased with the plant growth from 30 DAS to 90 DAS and thereafter, at 120DAS phenol content was decreased. Data presented in the table exhibited that, higher phenol content 0.71 mg/g and 0.70 mg/g on dry weight basis was present in resistant cultivars Bunty and H-1236 at 90 DAS, respectively. Whereas, leaves of HS-6 and RCH 134 BG-II of disease grade-6 showed lowest phenol content (0.25 mg/g in each) at 90DAS. It was observed from the data that plants with highest disease grade (grade-6) of HS-6 and RCH 134 BG-II showed lowest amount of phenol at any particular stage of the plant as compared to the resistant cultivars H-1236 and Bunty (Table 4.24 and Fig 4.5).

Table 4.24 Phenol content in resistant and susceptible cultivars of cotton at different period of time

Disease grade	Grades	Phenol (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	H-1236	0.65	0.68	0.70	0.66
0	HS-6	0.44	0.57	0.57	0.54
2	HS-6	0.36	0.42	0.44	0.39
4	HS-6	-	0.31	0.32	0.30
6	HS-6	-	0.24	0.25	0.20
0	Bunty	0.73	0.70	0.71	0.67
0	RCH-134 BGII	0.50	0.59	0.62	0.58
2	RCH-134 BGII	0.46	0.44	0.46	0.39
4	RCH-134 BGII	-	0.33	0.35	0.31
6	RCH-134 BGII	-	0.24	0.25	0.22
CD (at 5%)		**0.05**	**0.09**	**0.03**	**0.03**
CV (%)		**6.54**	**11.31**	**3.61**	**4.38**

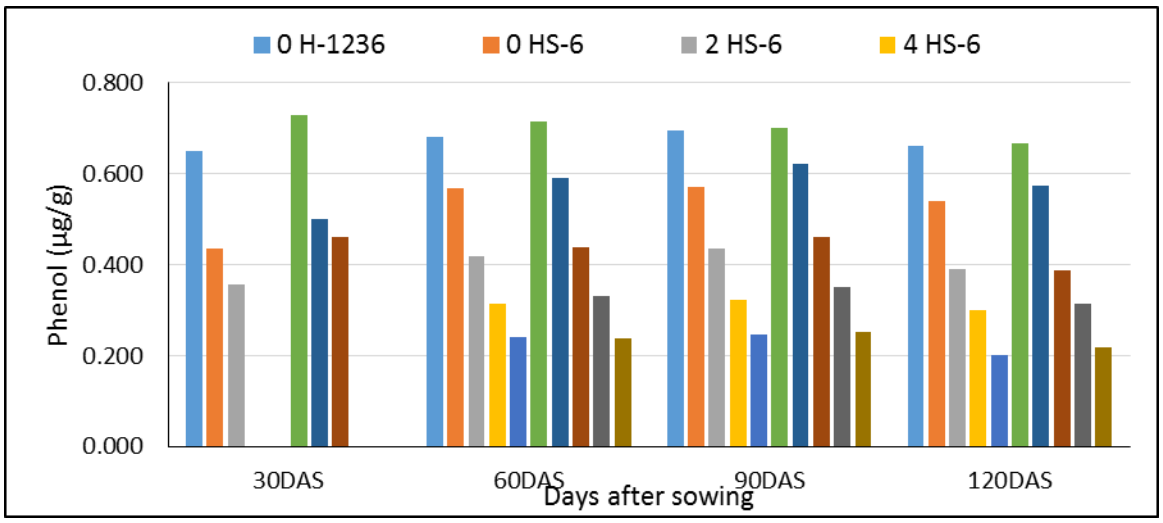

Fig 4.5 Phenol content in cotton cultivars at 30, 60, 90 and 120 DAS

4.3.2 Sugar (total and reducing)

Data in Table 4.25 and Fig 4.6 revealed that the maximum total sugar (14.9 mg/g on dry weight basis) was observed in resistant plants (grade-0) at 90 DAS in RCH 134 BG-II cultivar. Whereas, minimum sugar 1.5 mg/g was observed in leaves of HS-6 at 60 DAS in plants with disease grade-6. Sugar content did not show any specific trend either with growth stage or with disease grade of susceptible and resistant cultivars (Table 4.25 and Fig 4.6). However, data presented in table showed that near maturity i.e. at 120 DAS reduction in sugar content was observed in leaves from all grades.

Table 4.25 Total sugar content in resistant and susceptible cultivars of cotton at different period of time

Disease grade	Grades	Total sugar (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	HS-6	3.9	3.8	7.5	7.4
2	HS-6	2.6	4.7	3.4	3.3
4	HS-6	-	7.5	6.4	6.1
6	HS-6	-	1.5	7.1	7.0
0	RCH-134 BGII	13.5	11.5	14.9	14.6
2	RCH-134 BGII	9.2	9.9	2.4	2.3
4	RCH-134 BGII	-	8.4	8.3	8.2
6	RCH-134 BGII	-	6.8	6.5	6.4
0	H-1236	3.7	5.3	7.8	7.7
0	Bunty	8.9	11.2	2.0	1.9
CD (at 5%)		**0.13**	**1.35**	**0.29**	**0.42**
CV (%)		**1.27**	**11.07**	**2.56**	**3.70**

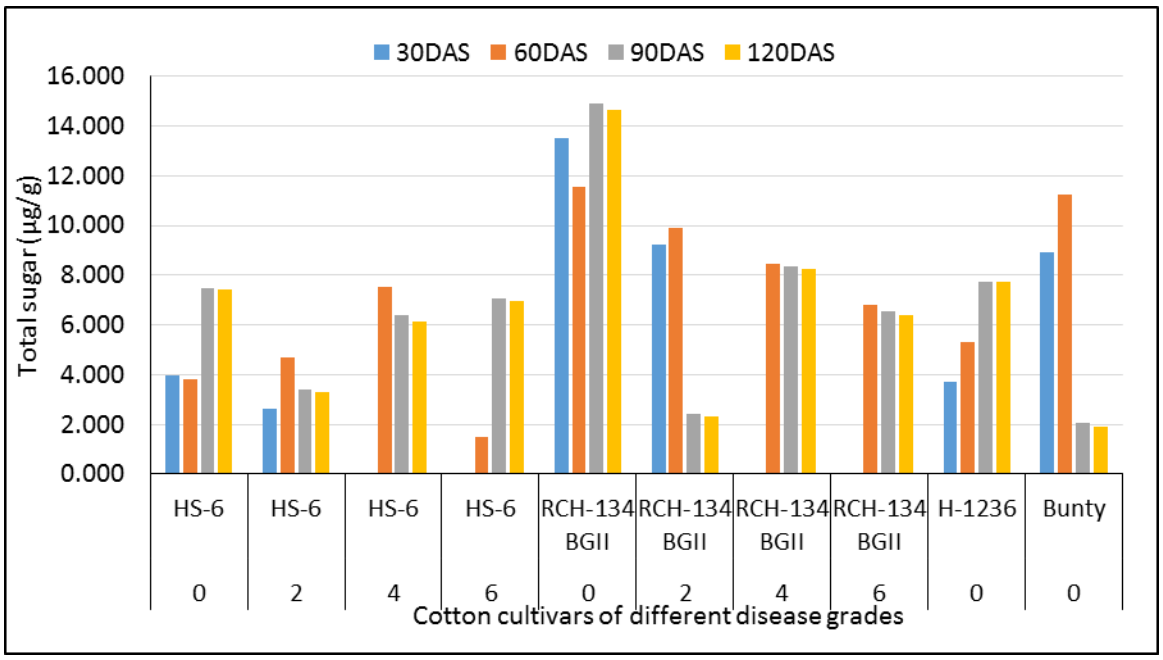

Fig 4.6 Sugar (total) content in cotton cultivars at 30, 60, 90 and 120 DAS

Similarly, perusal of data in Table 4.26 and Fig 4.7 exhibited that reducing sugar showed the similar trend as for the total sugar content. Maximum sugar (1.67 mg/g) was observed in healthy plants of RCH 134 BG II at 90 DAS. Whereas, lowest reducing sugar content (0.07 mg/g) was observed from highly diseased (grade-6) plants of HS-6 at 60 DAS. The reducing sugar content did not show any specific trend with the age and resistance of plant.

Table 4.26 Reducing sugar content in resistant and susceptible cultivars of cotton at different period of time

Disease grade	Grades	Reducing sugar (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	HS-6	0.27	0.25	0.72	0.65
2	HS-6	0.24	0.72	0.58	0.53
4	HS-6	-	0.36	0.19	0.18
6	HS-6	-	0.07	0.67	0.55
0	RCH-134 BGII	1.49	1.24	1.67	1.55
2	RCH-134 BGII	0.94	1.03	0.07	0.06
4	RCH-134 BGII	-	0.84	0.83	0.75
6	RCH-134 BGII	-	0.64	0.60	0.51
0	H-1236	0.24	0.44	0.76	0.74
0	Bunty	0.31	0.60	0.82	0.77
CD (at 5%)		**0.02**	**0.17**	**0.04**	**0.03**
CV (%)		**2.09**	**16.18**	**3.03**	**2.56**

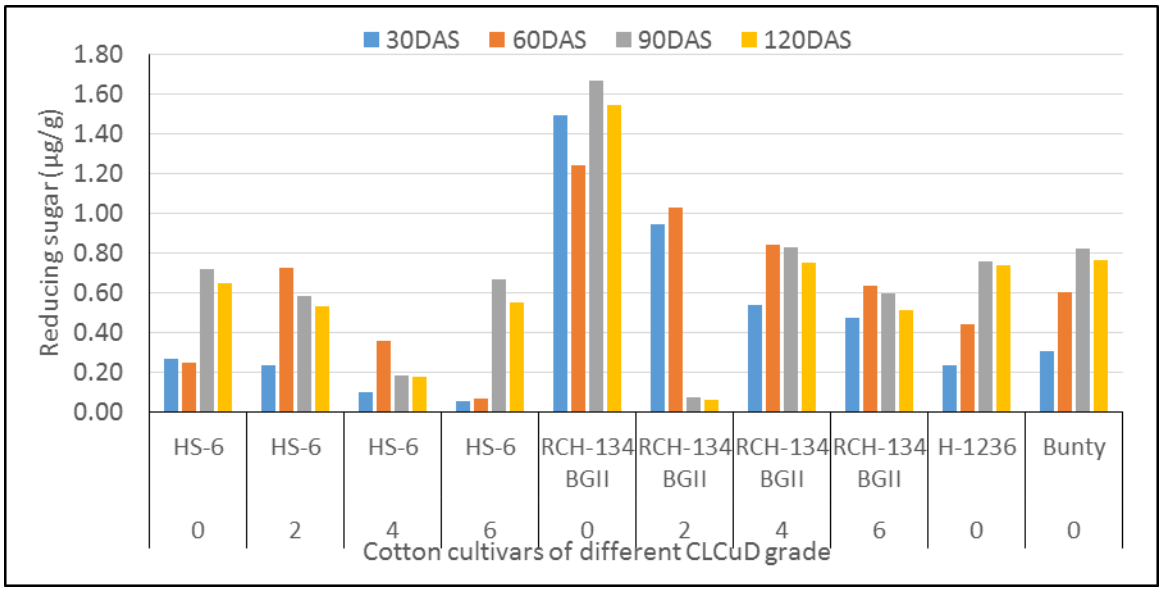

Fig 4.7 Sugar (reducing) content in cotton cultivars at 30, 60, 90 and 120 DAS

4.3.3 Gossypol

Data presented in Table 4.27 and Fig 4.8 exhibited that the increase in gossypol content was observed up to 90 DAS in all the cultivars. However, the gossypol content not showed any trend of change in concentration of gossypol in susceptible and resistant plants. Maximum gossypol content 0.75 mg/g was observed in susceptible cultivar RCH 134 BG-II at disease grade-4, followed by the resistant cultivar Bunty (0.74 mg/g), healthy leaves of RCH 134 BG-II (0.72 mg/g) and HS-6 (0.72 mg/g) of disease grade-4 at 90 DAS. Minimum gossypol was observed in plants with disease grade-6 (0.47 mg/g and 0.53 mg/g in HS-6 and RCH 134 BG-II, respectively. Therefore, the perusal of data from the table revealed that the change in concentration of gossypol was not affected by the severity of disease.

Table 4.327 Gossypol content in resistant and susceptible cultivars of cotton at different period of time

Disease grade	Cultivars	Gossypol (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	HS-6	0.56	0.56	0.65	0.58
2	HS-6	0.52	0.52	0.62	0.58
4	HS-6	-	0.53	0.72	0.70
6	HS-6	-	0.45	0.47	0.45
0	RCH-134 BGII	0.63	0.66	0.72	0.69
2	RCH-134 BGII	0.51	0.53	0.66	0.64
4	RCH-134 BGII	-	0.55	0.75	0.75
6	RCH-134 BGII	-	0.54	0.53	0.52
0	H-1236	0.62	0.60	0.62	0.61
0	Bunty	0.70	0.70	0.74	0.71
CD (at 5%)		**0.01**	**0.02**	**0.03**	**0.04**
CV (%)		**1.04**	**2.27**	**2.82**	**3.31**

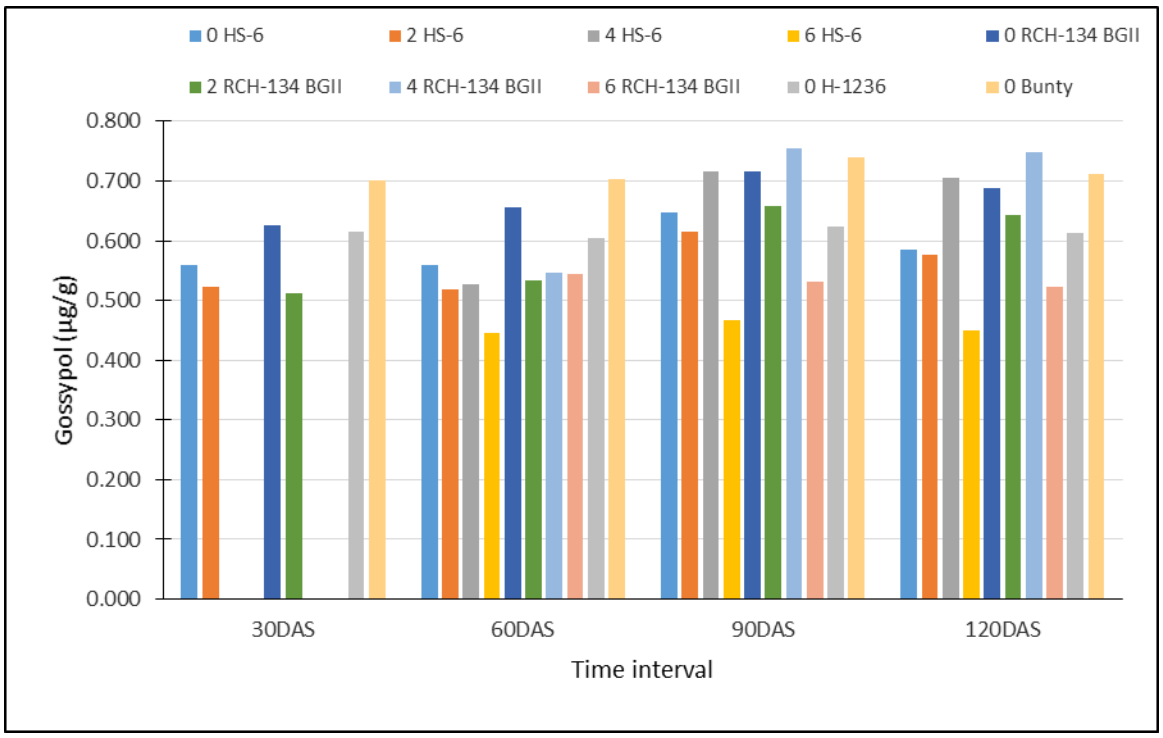

Fig 4.8 Gossypol content in cotton cultivars at 30, 60, 90 and 120 DAS

4.3.5 Tannin

Perusal of data from Table 4.28 and Fig 4.9 exhibited that the overall tannin content was increased up to 60 DAS in all the cultivars, whereas, at later stages (90 and 120 DAS) leaves showed decreasing trend in tannin content in all the cultivars. Maximum tannin content 0.93 mg/g on dry weight basis was observed in RCH 134 BG-II at 60 DAS in plant with no disease, which was closely followed by tannin content in resistant cultivar Bunty (0.90 mg/g). Plants with lower disease grade showed higher values of tannin content as compared to the plants with higher disease grade. Data presented in the table revealed that healthy leaves of HS-6 extracted high tannin content 0.76 mg/g, whereas, highly diseased plants (grade-6) showed 0.47 mg/g tannin at 60 DAS. Similarly, RCH 134 BG-II plants showed 0.93 mg/g tannin in healthy leaves and 0.61 mg/g tannin in highly diseased (grade-6) plants.

Data presented in Table 4.28 revealed that tannin content of susceptible cultivars (HS-6 and RCH 134 BG-II) showed decreasing trend with the increase in disease severity (disease grade index). Resistant cultivars H-1236 and Bunty also showed high tannin content during all the growth stages. The overall tannin content increased up to 60 DAS in all the cultivars and thereafter, it decreased at 90 and 120 DAS.

Table 4.28 Tannin content in resistant and susceptible cultivars of cotton at different period of time

Disease grade	Grades	Tannin (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	H-1236	0.78	0.76	0.51	0.45
0	HS-6	0.75	0.77	0.55	0.47
2	HS-6	0.59	0.58	0.23	0.14
4	HS-6	-	0.55	0.17	0.08
6	HS-6	-	0.47	0.29	0.22
0	Bunty	0.78	0.90	0.50	0.47
0	RCH-134 BGII	0.79	0.93	0.71	0.43
2	RCH-134 BGII	0.69	0.76	0.43	0.24
4	RCH-134 BGII	-	0.72	0.20	0.19
6	RCH-134 BGII	-	0.61	0.24	0.39
CD (at 5%)		**0.03**	**0.03**	**0.02**	**0.06**
CV (%)		**2.45**	**2.12**	**2.26**	**10.26**

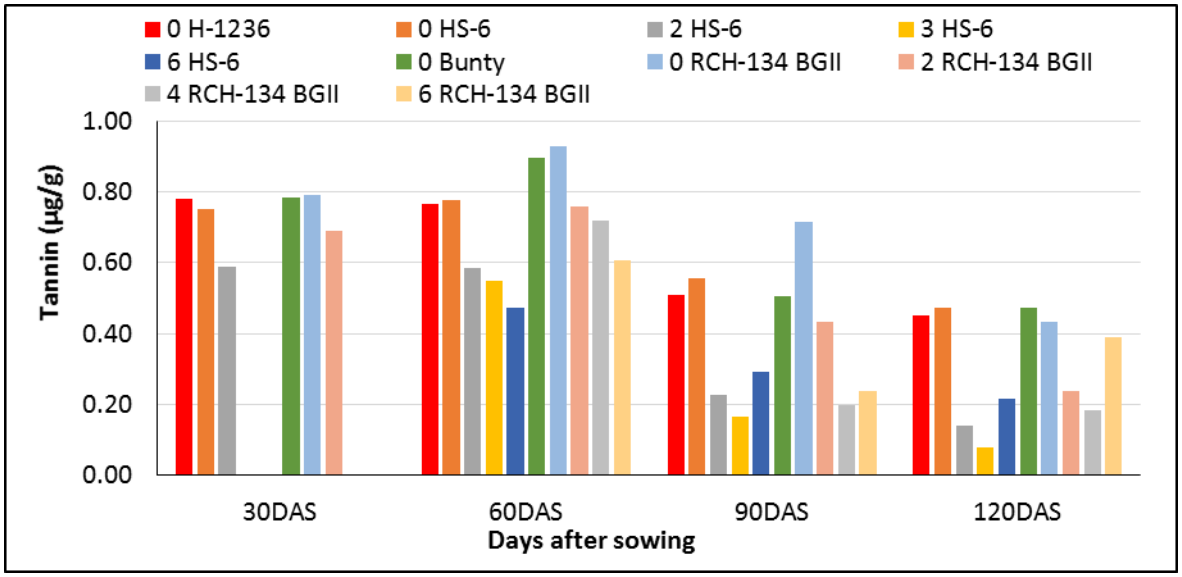

Fig 4.9 Tannin content in cotton cultivars at 30, 60, 90 and 120 DAS

4.3.4 Protein

Data presented in Table 4.29 and Fig 4.10 revealed that that maximum protein content (26.3 mg/g) was observed in resistant cultivar Bunty at 90 DAS, followed by H-1236 with 25.1 mg/g protein. Leaves from healthy plants of susceptible cultivars also showed increased protein

content. Protein content of RCH 134 BG-II and HS-6 was 24.3 mg/g and 21.2 mg/g at 90 DAS. The data presented in the table revealed that in susceptible cultivars, plants with lower disease grade extracted more protein as compared to plants with higher disease index. Plants with highest disease score (grade-6) have shown lower protein content. Data presented in Table 4.29 and Fig 4.10 exhibited that lowest protein content 9.4 mg/g was observed in cv. HS-6 followed by RCH 134 BG-II (10.5 mg/g) at 120 DAS.

Table 4.29 Protein content in resistant and susceptible cultivars of cotton at different period of time

Treatments	Cultivars	Protein (mg/g dry weight basis)			
		30DAS	60DAS	90DAS	120DAS
0	HS-6	8.8	15.1	21.2	20.7
2	HS-6	8.3	13.9	19.4	18.5
4	HS-6	-	12.5	15.0	14.0
6	HS-6	-	9.7	10.8	9.4
0	RCH-134 BGII	9.3	15.9	24.3	21.2
2	RCH-134 BGII	8.8	14.7	20.8	19.1
4	RCH-134 BGII	-	12.8	15.8	14.5
6	RCH-134 BGII	-	10.5	12.0	10.5
0	H-1236	9.9	15.3	25.1	24.3
0	Bunty	10.3	16.0	26.3	24.8
CD (at 5%)		**0.31**	**0.32**	**0.33**	**0.36**
CV (%)		**2.24**	**1.36**	**1.01**	**1.16**

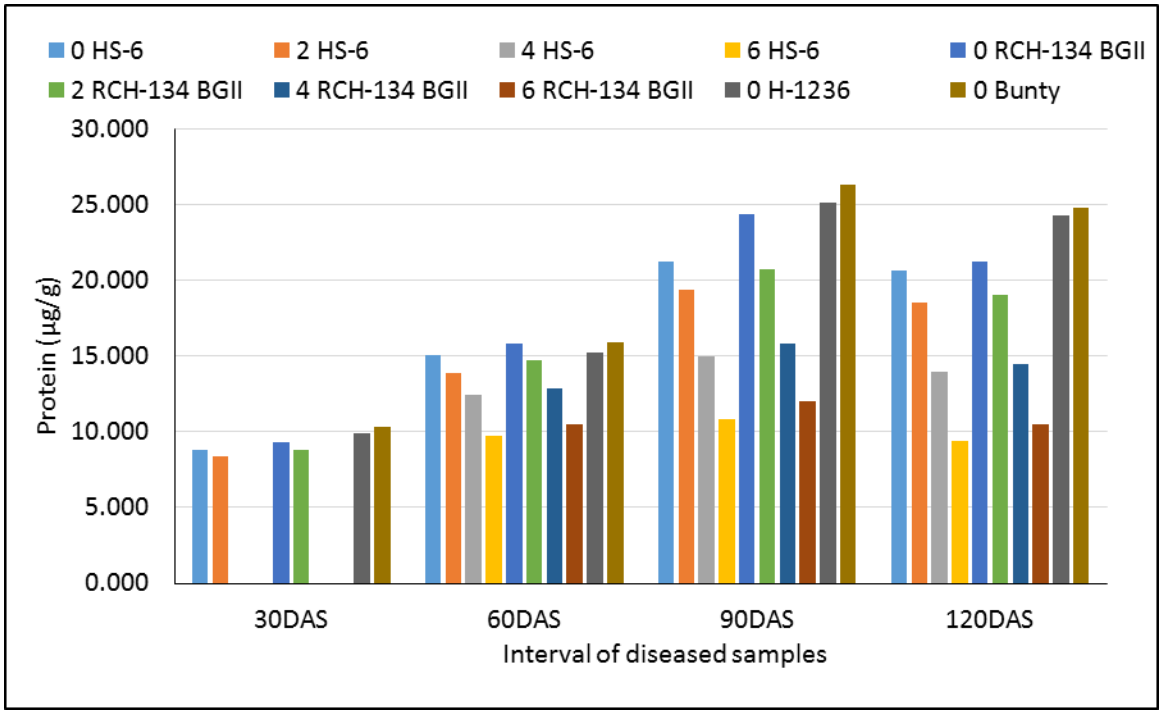

Fig 4.10 Protein content in cotton cultivars at 30, 60, 90 and 120 DAS

4.3.1 Chlorophyll (a and b)

Chlorophyll determination test was conducted on freshly harvested leaves at different growth stages. Perusal of data in Table 4.30 and Fig 4.11 exhibited that Chlorophyll-a (Chl-a) increased with increase in disease grade and crop growth stages.

Table 4.30 Chlorophyll-a and b content in resistant and susceptible cultivars of cotton at different period of time

Grades	Cultivars	Chlorophyll-a (mg/g fresh weight basis)				Chlorophyll-b (mg/g fresh weight basis)			
		30DAS	60DAS	90DAS	120DAS	30DAS	60DAS	90DAS	120DAS
0	HS-6	112.3	113.0	109.7	106.9	58.2	92.5	112.0	98.4
2	HS-6	168.4	172.7	167.2	161.6	97.2	117.7	147.7	145.2
4	HS-6	-	192.6	192.2	184.7	-	157.3	177.0	175.0
6	HS-6	-	284.5	342.8	332.5	-	163.1	172.5	167.7
0	RCH-134 BGII	90.4	90.8	122.8	119.3	63.6	120.2	131.9	125.4
2	RCH-134 BGII	103.8	102.2	159.4	155.2	56.0	131.8	157.3	155.4
4	RCH-134 BGII	-	114.2	171.3	168.8	-	143.9	162.9	159.6
6	RCH-134 BGII	-	170.8	299.1	296.6	-	153.7	132.9	133.4
0	H-1236	223.8	99.1	116.2	114.9	50.2	127.2	147.7	127.4
0	Bunty	245.8	112.2	114.8	113.4	53.2	116.3	145.4	139.3
CD (at 5%)		**36.4**	**21.2**	**24.7**	**22.5**	**14.1**	**33.8**	**37.4**	**47.9**
CV (%)		**13.2**	**10.8**	**15.5**	**17.4**	**14.6**	**8.2**	**11.4**	**18.3**

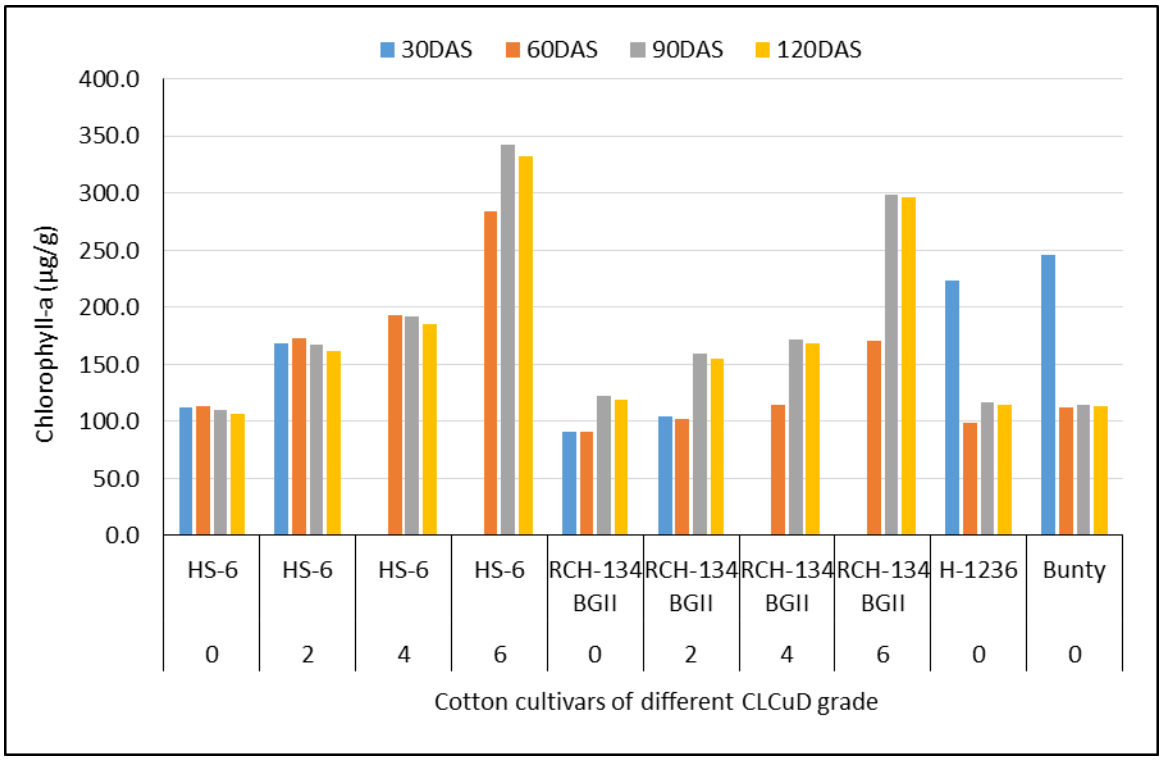

Fig 4.11 Chlorophyll –a content in cotton cultivars at 30, 60, 90 and 120 DAS

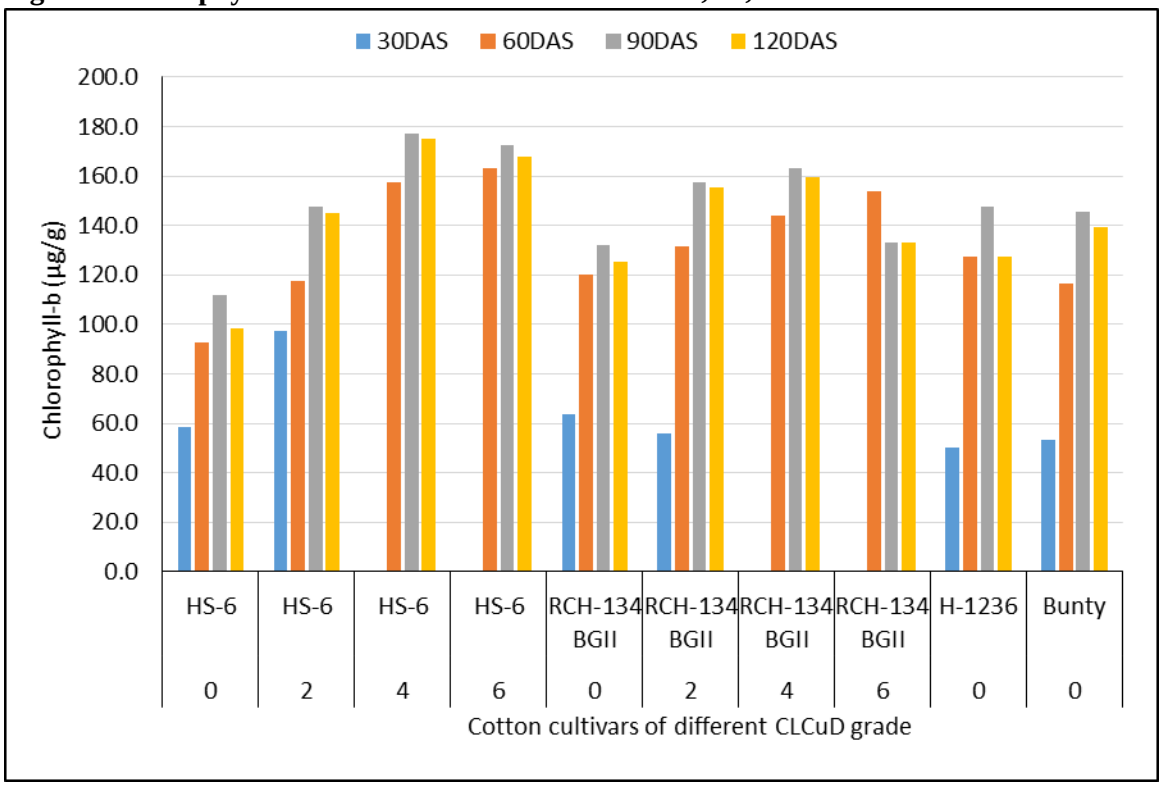

Fig 4.12 Chlorophyl-b content in cotton cultivars at 30, 60, 90 and 120 DAS

Chl-a increased up to 90 DAS in CLCuD susceptible cultivars (HS-6 and RCH 134 BG-II). Maximum Chl-a 342.8mg/g was observed in HS-6 on fresh weight basis, followed by RCH 134 BG-II (299.1 mg/g) from highly diseased plants (grade-6) at 90 DAS. Whereas, Chl-a in resistant cultivars H-1236 and Bunty was observed minimum at 30 DAS (223.8 mg/g and 245.8 mg/g, respectively). Perusal of data in Table 4.30 and Fig 4.12 revealed that the chlorophyll-b (Chl-b) was lowest at 30 DAS after sowing in all the cultivars and increased with the time up to 90 DAS. Data also revealed that the total amount of Chl-a, was remained higher than Chl-b at a particular stage of plant.

4.3.7 Peroxidase

Perusal of data in Table 4.31 and fig. 4.13 revealed that resistant cultivars H-1236 and Bunty have shown high peroxidase (PO) activity as compared to the diseased plants of susceptible cultivars. Data exhibited that at 30 DAS peroxidase activity was 1.8 to 34.5 min $^{-1}$/g of leaf in H-1236 and 1.7 to 34.8 min $^{-1}$/g in Bunty. Similarly, healthy leaves of susceptible cultivars HS-6 and RCH 134 BG-II also showed more enzyme activity (1.7 to 34.0 min $^{-1}$/g and 2.1 to 33.1 min $^{-1}$/g, respectively). Plants with higher disease of susceptible cultivar showed less PO activity as compared to healthy and resistant plants. Perusal of data in the table revealed that the highest PO activity in the susceptible cultivars was observed at 30DAS. Peroxidase activity in HS-6 was 2.8 to 22.6 min $^{-1}$/g and 3.1 to 20.6 min $^{-1}$/g in RCH 134 BG-II at 30DAS. Leaf samples of all plant stage (60, 90 and 120 DAS) showed similar trend of enzyme activity for resistant and susceptible cultivars. Overall PO activity was observed increasing up to 90 DAS and decreased at 120 DAS.

4.3.8 Polyphenol oxidase

Perusal of data in Table 4.32 and Fig. 14 exhibited that resistant cultivars showed high polyphenol oxidase (PPO) activity as compared to the susceptible cultivars. Polyphenol oxidase activity was observed maximum in the resistant cultivar Bunty (10.2 to 18.9 min $^{-1}$/g) followed by H-1236 (10.1 to 18.3 min $^{-1}$/g) of fresh leaves at 30 DAS. Healthy leaves (grade-0) of susceptible cv. RCH 134 BG-II also showed high PPO activity (10.2 to 17.4 min $^{-1}$/g) followed by HS-6 (from 9.5 to 16.8 min $^{-1}$/g). Data presented in table revealed that plants with higher disease grade/severity showed less PPO activity than of the healthy and resistant plants. The trend in decrease in PPO activity with the increase in CLCuD disease was observed in plants for all the growth stages (60, 90 and 120 DAS).

Table 4.31 Peroxidase (PO) activity in diseased and healthy plants of resistant and susceptible cultivars of cotton

Disease Grade	Cultivars	Peroxidase (Δ430 nm min^{-1}/g leaf tissue)																			
		30DAS					60DAS					90DAS					120DAS				
		Time progress in minute					Time progress in minute					Time progress in minute					Time progress in minute				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
0	HS-6	1.7	7.5	16.4	25.3	34.0	1.3	6.2	14.2	21.7	29.6	5.2	11.7	19.6	27.1	35.0	1.3	7.9	15.8	23.3	31.2
2	HS-6	2.8	5.9	13.8	18.0	22.6	1.8	6.9	7.7	9.7	11.3	5.6	12.4	13.2	15.2	16.8	1.8	8.6	9.4	11.4	13.0
4	HS-6	0.0	0.0	0.0	0.0	0.0	4.7	5.7	6.1	6.3	6.5	8.5	11.2	11.6	11.8	12.0	4.7	7.3	7.8	8.0	8.2
6	HS-6	0.0	0.0	0.0	0.0	0.0	9.1	9.9	10.4	10.6	10.8	12.9	15.4	15.9	16.1	16.3	9.1	11.6	12.1	12.3	12.5
0	RCH-134 BGII	2.1	6.6	21.0	26.6	33.1	2.3	7.1	15.8	25.6	31.0	6.1	12.6	21.3	31.0	36.5	2.3	8.8	17.5	27.2	32.7
2	RCH-134 BGII	3.1	5.3	12.3	16.2	20.6	5.9	6.1	6.3	6.4	6.6	9.7	11.6	11.8	11.9	12.1	5.9	7.8	8.0	8.1	8.3
4	RCH-134 BGII	0.0	0.0	0.0	0.0	0.0	5.3	5.6	5.8	6.1	6.2	9.1	11.1	11.3	11.5	11.7	5.3	7.3	7.5	7.7	7.9
6	RCH-134 BGII	0.0	0.0	0.0	0.0	0.0	9.8	10.1	10.5	10.8	11.0	13.6	15.6	16.0	16.3	16.5	9.8	11.8	12.2	12.5	12.7
0	H-1236	1.8	9.4	19.1	27.8	34.5	2.2	5.9	16.5	24.2	30.1	6.0	11.4	22.0	29.7	35.6	2.2	7.6	18.2	25.9	31.7
0	Bunty	1.7	9.8	18.5	29.3	34.8	3.1	7.2	16.6	26.4	31.8	6.9	12.7	22.1	31.9	37.3	3.1	8.9	18.3	28.1	33.5
	CD (at 5%)	**0.4**	**0.9**	**2.7**	**2.5**	**1.8**	**1.0**	**0.8**	**1.0**	**1.5**	**1.3**	**1.0**	**0.8**	**1.0**	**1.5**	**1.3**	**1.0**	**0.8**	**1.0**	**1.5**	**1.3**

Table 4.32 Polyphenol oxidase (PPO) activity in diseased and healthy plants of resistant and susceptible cultivars of cotton

Disease Grade	Cultivars	Polyphenol oxidase (Δ430 nm min^{-1}/g leaf tissue)																			
		30DAS					60DAS					90DAS					120DAS				
		Time progress in minute					Time progress in minute					Time progress in minute					Time progress in minute				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
0	HS-6	9.5	10.8	13.4	15.5	16.8	8.3	9.8	11.7	13.9	15.6	7.1	8.7	10.1	12.1	13.9	16.0	25.8	41.5	60.2	68.3
2	HS-6	8.4	9.0	9.9	10.8	12.3	7.5	8.1	8.8	9.7	11.0	6.6	7.3	8.1	8.9	10.1	14.3	23.0	28.6	46.1	61.2
4	HS-6	0.0	0.0	0.0	0.0	0.0	6.5	7.2	8.2	8.9	9.7	5.5	6.1	7.2	7.8	8.5	10.1	17.8	23.3	40.4	51.4
6	HS-6	0.0	0.0	0.0	0.0	0.0	5.6	6.8	7.7	8.2	8.9	4.3	6.0	6.7	7.2	7.6	7.6	10.8	14.1	24.9	32.8
0	RCH-134 BGII	10.2	11.0	14.6	16.0	17.4	8.8	10.3	12.7	13.6	14.8	7.7	9.7	10.8	11.2	12.3	17.1	28.6	45.4	61.9	70.5
2	RCH-134 BGII	9.4	10.3	11.0	12.0	12.9	8.3	9.4	10.4	11.2	12.1	7.1	8.4	9.7	10.3	11.3	15.6	25.1	32.1	48.8	63.0
4	RCH-134 BGII	0.0	0.0	0.0	0.0	0.0	7.5	8.2	9.4	10.1	11.3	6.4	7.2	8.4	9.2	10.2	11.5	19.5	24.5	43.4	58.5
6	RCH-134 BGII	0.0	0.0	0.0	0.0	0.0	6.4	7.1	7.9	8.6	9.4	5.8	6.4	7.4	7.8	8.6	8.7	11.3	15.5	26.3	37.1
0	H-1236	10.1	11.3	14.9	16.8	18.3	8.8	10.4	12.8	14.8	16.5	7.5	9.4	10.7	12.8	14.6	17.3	26.9	43.0	61.8	69.5
0	Bunty	10.2	11.9	16.3	17.4	18.9	8.7	11.1	13.9	15.3	17.1	7.7	10.2	11.5	13.2	15.3	18.0	28.4	47.1	69.1	71.8
	CD (at 5%)	**0.4**	**0.5**	**0.6**	**0.6**	**0.6**	**0.6**	**0.5**	**0.4**	**0.4**	**0.5**	**0.5**	**0.7**	**17.2**	**2.6**	**3.6**	**0.4**	**0.3**	**0.8**	**1.0**	**1.1**

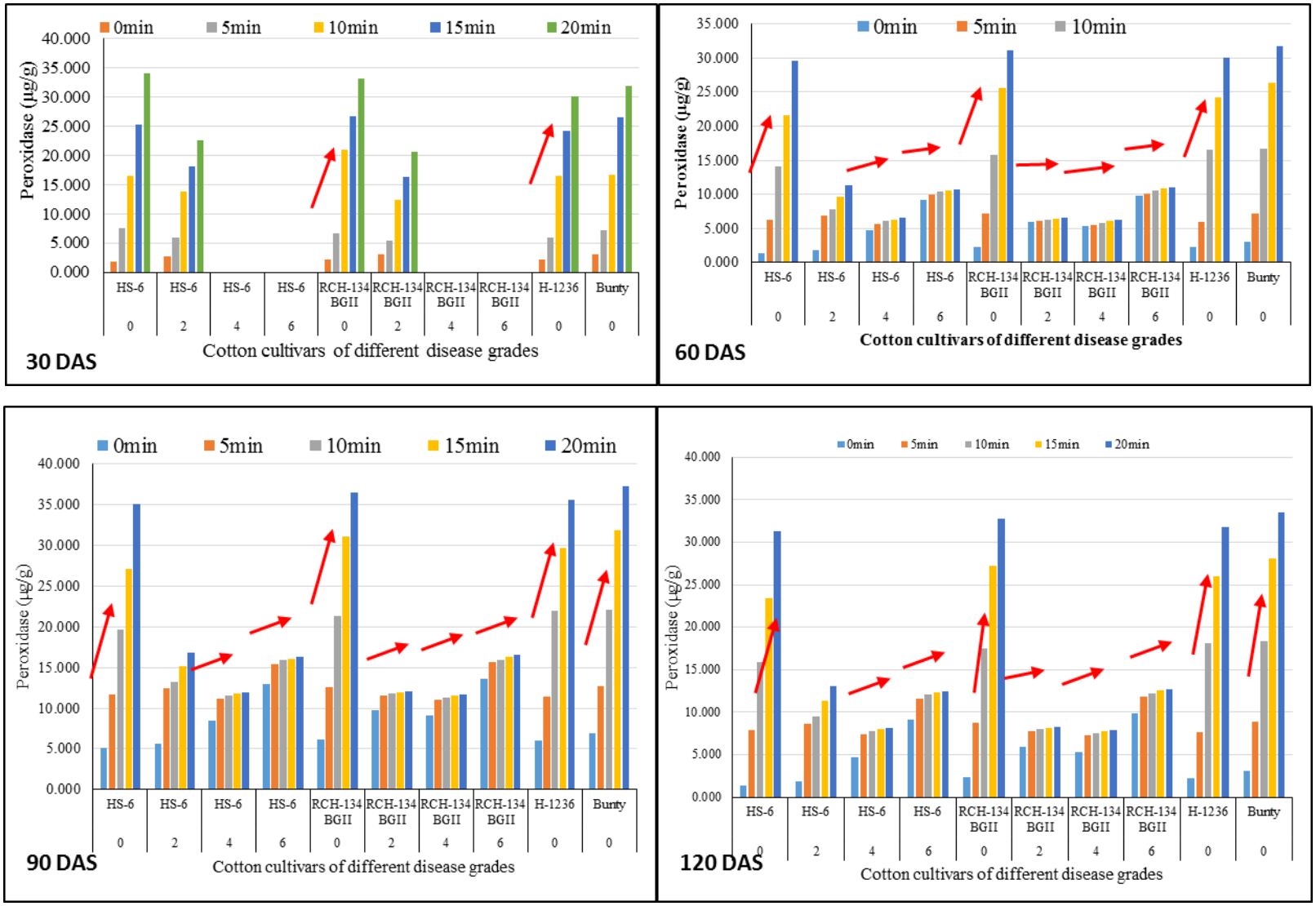

Fig 4.13 Peroxidase (PO) activity in cotton cultivars at 30, 60, 90 and 120 DAS

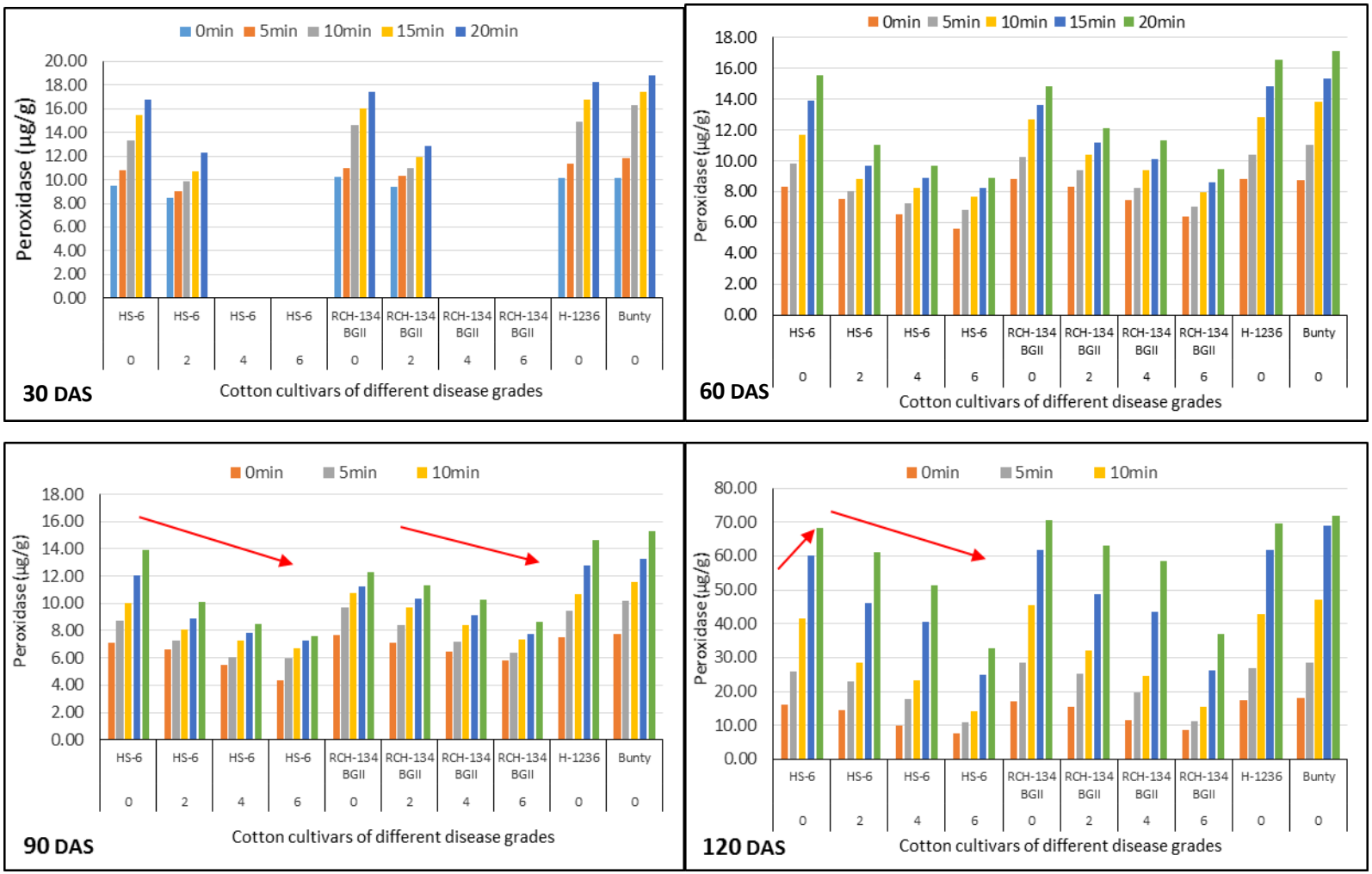

Fig 4.14 Polyphenol oxidase (PPO) activity in cotton cultivars at 30, 60, 90 and 120 DAS

4.4 Molecular studies

4.4.1 Qualitative and Quantitative Evaluation of DNA samples

Isolated DNA contained excessive polysaccharides and polyphenolics therefore, it was essentially processed to get polysaccharide and polyphenolics free DNA and then qualitatively and quantitatively analyzed using agarose gel electrophoresis. The concentration of DNA samples was quantified by visual assessment of band intensity in comparison with Lambda DNA of 50 ng/µl concentration in 0.8% agarose gel (Plate 4.3). The quality was found to be fairly good for further processing. Samples having DNA concentration >150 ng were used further.

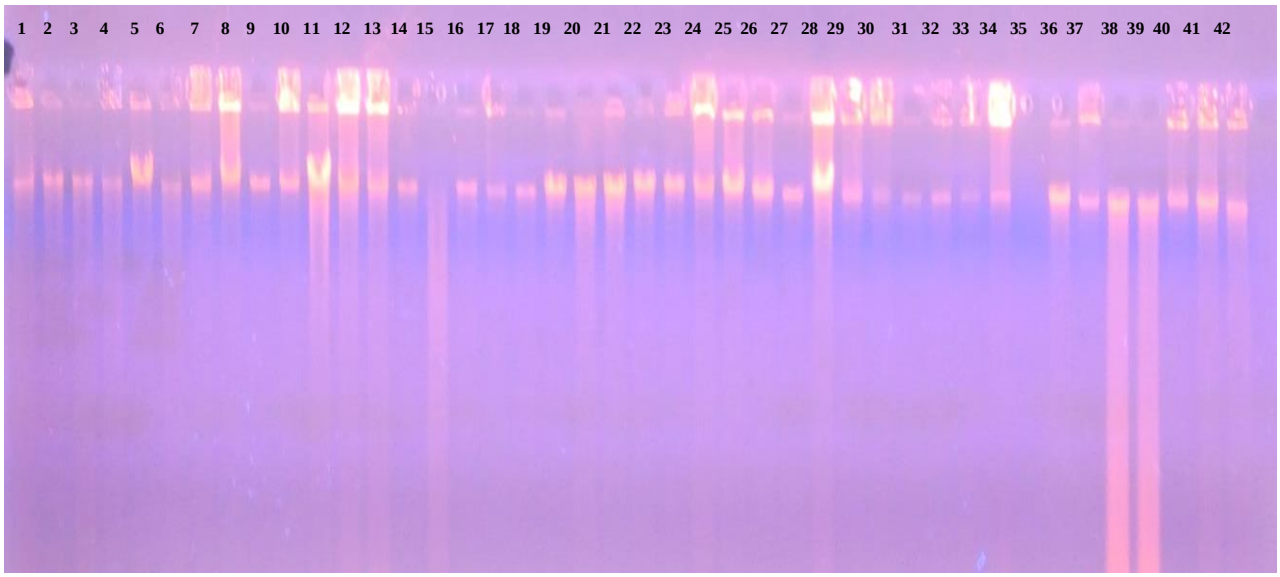

Plate 4.3. Agarose gel (0.8%) showing genomic DNA from different cotton leaf samples

4.4.2 Standardization of PCR Amplification

For CLCuV coat protein gene specific primers, PCR amplification conditions *viz*. concentration of template DNA, primers, $MgCl_2$, Taq polymerase and annealing temperatures were standardized. Varying concentrations of template DNA (50-150 ng), Taq DNA polymerase (1.0-1.5 U), dNTPs (1-1.2 mM), primers (2-5 pM), $MgCl_2$ (1.5-5 mM), were standardized for a reaction volume of 15µl.

4.4.3 Optimization of PCR Amplification

Conditions Composition of different PCR components in reaction mixture for optimum amplification were standardized (Table 4.33). Reproducible and clear banding pattern for all three CLCuV coat protein gene specific primers (CtLCV) were obtained in a reaction mixture of composition tabulated in Table 4.33.

Tables 4.33 Optimized PCR conditions for CtLCV primers

Sr. no.	Component	Amount per reaction mixture (15µl)
1	Template DNA	1µl (100ng)
2	10X PCR buffer*	1.5µl
3	dNTPs	1.5µl (1mM)
4	Forward primer	0.75 µl (5pM)
5	Reverse primer	0.75 µl (5pM)
6	Taq polymerase	1.15 µl (1.15U)
7	Sterile distilled water	8.35 µl

*10X PCR buffer contains 1.5mM $MgCl_2$

Different temperatures and time intervals during denaturation, annealing, and extension steps were also optimized. An immense effect of annealing temperature on amplification was noticed. Different optimized temperatures specifically annealing temperature and time intervals of each step for different CtLCV primers have been enlisted in Table 4.34 (CtLCV#33 primer), Table 4.35 (CtLCV#45 primer) and Table 4.36 (CtLCV#55 primer):

Table 4.34Optimized temperature and time interval of CtLCV#33 primer

Sr.	Step	Temperature	Time interval
1	Initial denaturation	92ºC	3 minutes
2	Denaturation	91 ºC	45 seconds
3	Annealing	43.5 ºC	50 seconds
4	Extension	72 ºC	2 minutes
5	Final extension	72 ºC	5 minutes

Table 4.35 Optimized temperature and time interval of CtLCV#45 primer

Sr. no.	Step	Temperature	Time interval
1	Initial denaturation	92ºC	3 minutes
2	Denaturation	91 ºC	45 seconds
3	Annealing	49 ºC	50 seconds
4	Extension	72 ºC	2 minutes
5	Final extension	72 ºC	5 minutes

Table 4.36 Optimized temperature and time interval of CtLCV#55 primer

Sr.	Step	Temperature	Time interval
1	Initial denaturation	92ºC	3 minutes
2	Denaturation	91 ºC	50 seconds
3	Annealing	49 ºC	45 seconds
4	Extension	72 ºC	2 minutes
5	Final extension	72 ºC	5 minutes

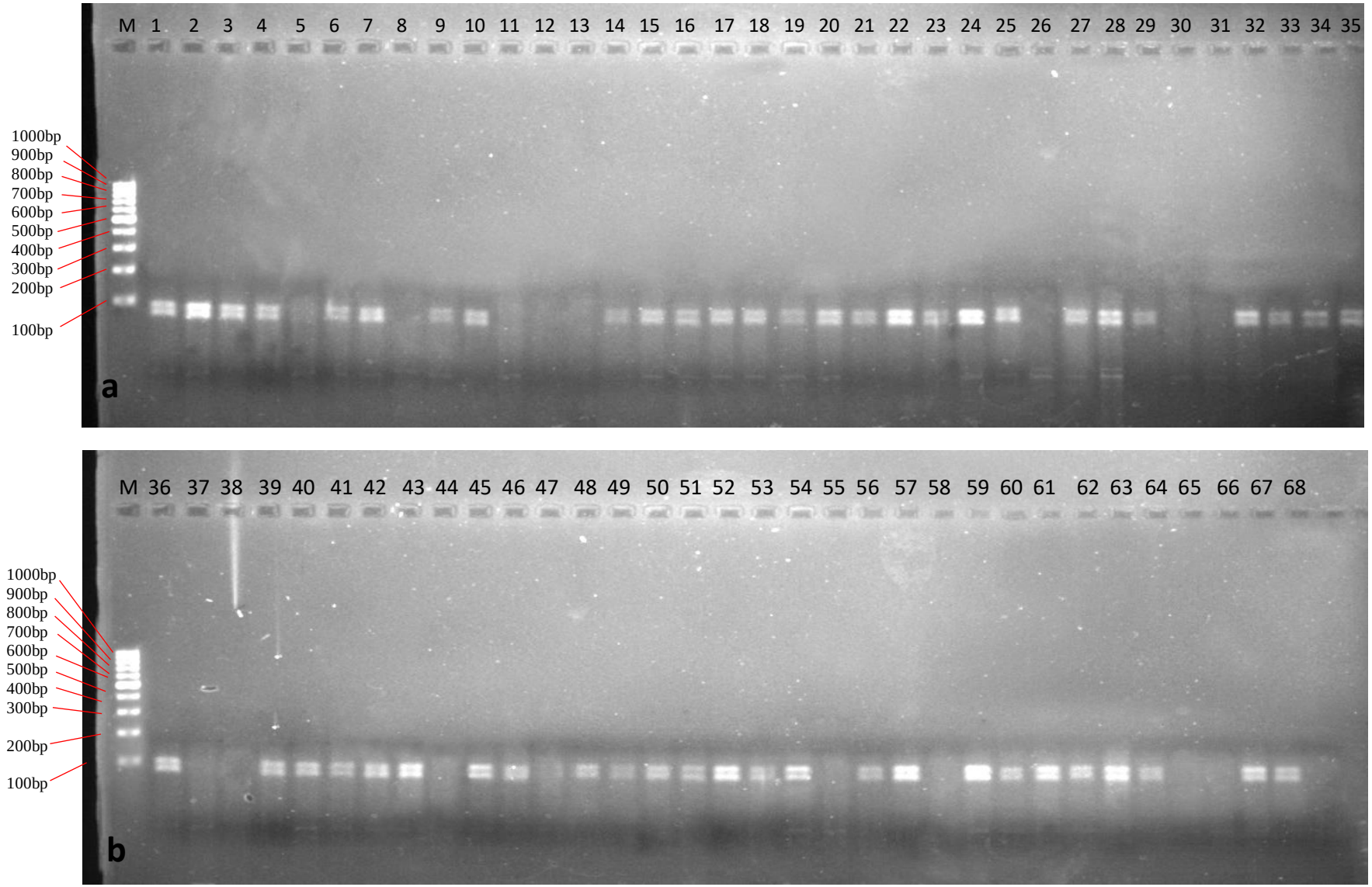

Plate 4.4a & b. Agarose gel showing genomic DNA from different cotton leaves sample against CtLCV # 33 marker

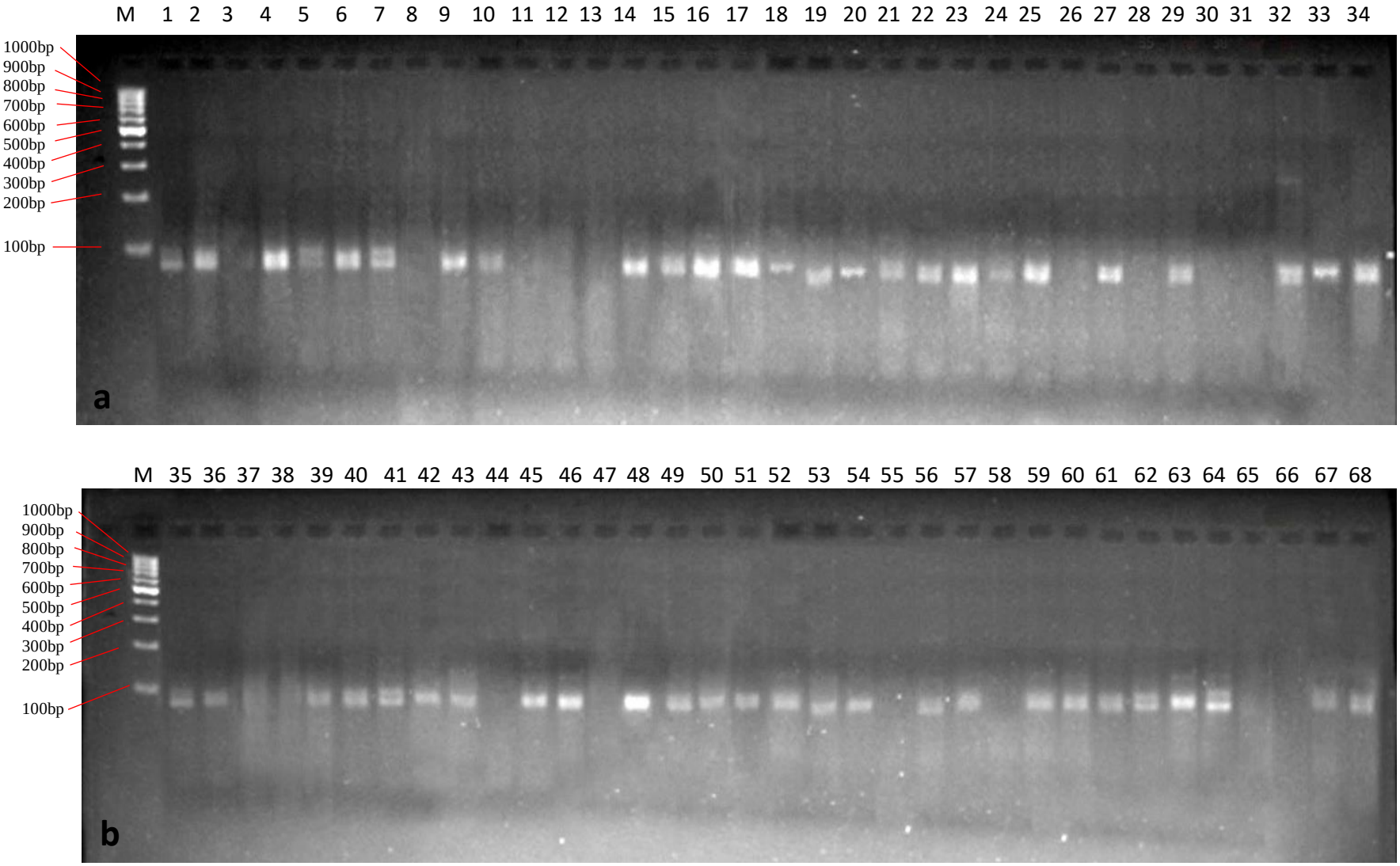

Plate 4.5a & b. Agarose gel showing genomic DNA from different cotton leaves sample against CtLCV # 45 marker

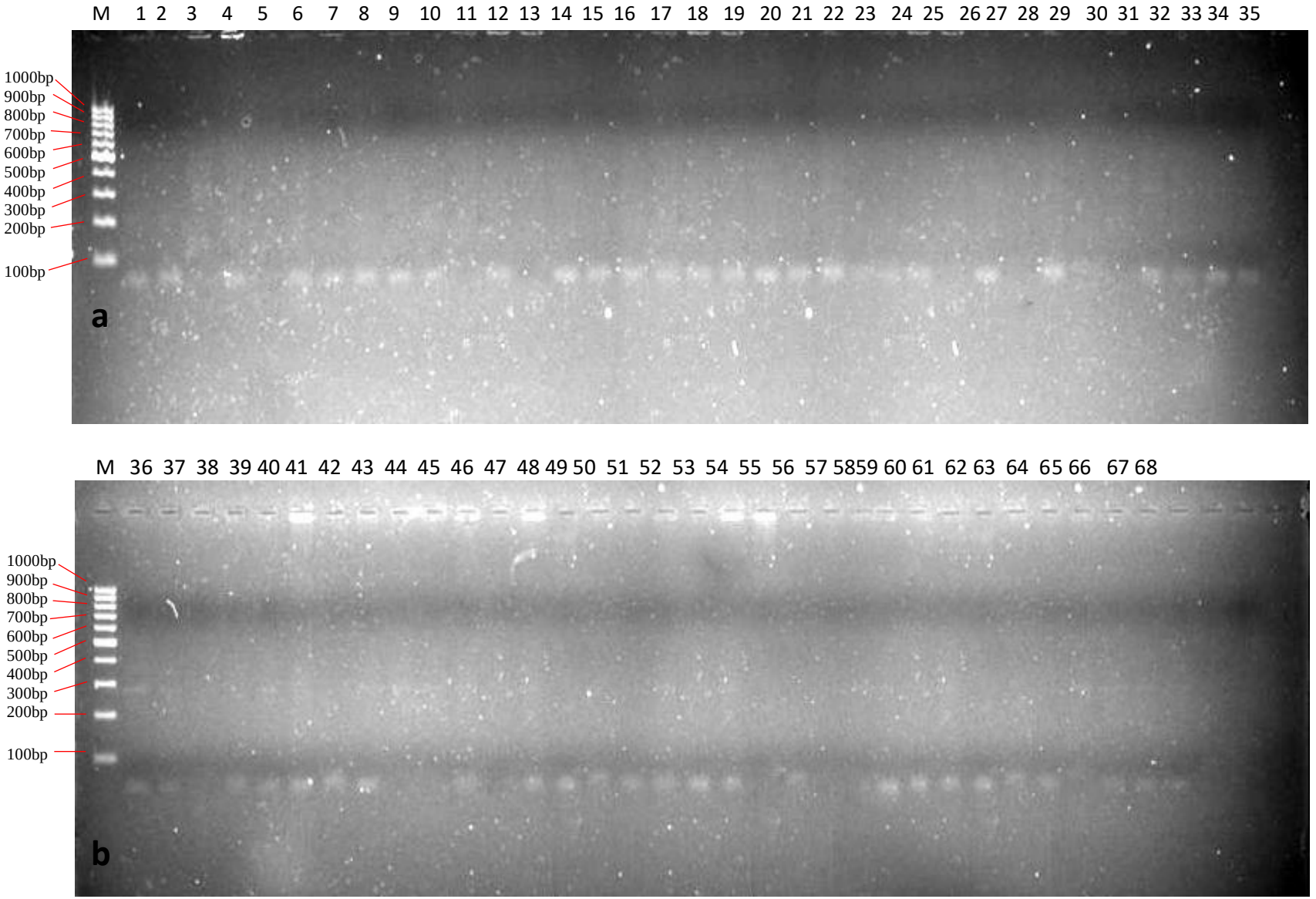

Plate 4.6a & b. Agarose gel showing genomic DNA from different cotton leaves sample against CtLCV # 55 marker

Table 4.37 Presence and absence of DNA bands in samples run on agarose gel against three different CLCuV specific primers

P-1		P-2		P-3		P-1,2		P-1,3		P-2,3		P-1,2,3		Absent	
S	**Place**	**S**	**Place**	**S**	**Place**	**S**	**Place**	**S**	**Place**	**S**	**Place**	**S**	**Place**	**S**	**Place**
3	Kaimari	5	Matersyam	8	Adampur	23	Nathusari	37	Badhra	14	Hansi	1	CCS HAU	11	Moda Khera
28	Jagmalwali	39	Jind	12	Channi Badi	24	Bhuna	44	Pathrala	27	Kalanwali	2	Chaudharywas	13	Balsamand
30	Rania	45	Kishanpur	38	Siwani	26	Odhan	47	Talwandi sabo	32	M.dabwali	4	Aryanagar	31	Jeevan Nagar
58	Khuiya sarbar	48	Rori			36	Bawanikhera	55	Abohar	60		6	Barwala	66	Mahiyanwali
		56	Kabarwala			46	Malaut	65	Jogiwala			7	Uklana		
		59	Kallar khera									9	Agroha		
		67	Pakka Sahrana									10	Bodha Hoshnak		
												15	Fatehabad		
												16	Hamjapur		
												17	Basin		
												18	Bhattukalan		
												19	Ding		
												20	Ratia		
												21	Kawalgarh		
												22	Tohana		
												25	Sirsa		
												29	Ellanabad		
												33	Abubshahar		
												34	Dudhiawali		
												35	Bhiwani		
												40	Narwana		
												41	Danoda Kalan		
												42	Unchana		
												43	Bathinda		
												49	Fazilka		
												50	Ganjuana		
												51	Kui Khera		
												52	Nihalkhera		
												53	Chuvadiawali		
												54	Panniwala		
												57	Pakki Tibbi		
												61	Sadhuwali		
												62	Sadul sahar		
												63	Lalgarh Jatan		
												64	Mahiyanwali		
												68	Rodawali		
Total															
4		**7**		**3**		**5**		**5**		**4**		**36**		**4**	

Where, P-1 = Primer CtLCV # 33, P-2 = CtLCV # 45 and P-3 = CtLCV # 55; S= Sample number

Final amplification was carried out in different DNA samples having sequences of cotton leaf curl virus coat protein gene specific primers. Amplification of different isolates of CLCuV with three primers CtLCV#33, CtLCV#45 and CtLCV#55 are presented in plate no. 4.4a & b, Plate no. 4.5a & b and Plate no. 4.6a & b, respectively.

Results from plates (4.4 to 4.6) showed monomorphic bands and indicated similar molecular weight of genomic DNAs of isolated from CLCuD samples from Haryana, Punjab and Rajasthan. However, the results showed variations in presence and absence of DNA in the various samples after they were run against coat protein specific markers CtLCV # 33, CtLCV # 45 and CtLCV # 55.

CLCuV isolates of cotton leaf samples from Kaimari (S.N.-3), Jagmaliwali (28), Rania (30) and Khuiya (58) showed bands with primer-1 (#33) only. Isolates of seven places Matrasyam (5), Jind (39), Kishanpur (45), Rori (48), Kabarwala (56), Kallar (59) and Pakka (67) showed amplification with primer-2 (#45) only. The isolates of Adampur (8), Channi badi (12) and Siwani (38) showed amplification with primer-3 (#55) only.

Leaves sample of five locations, Nathusari chopta (23), Bhuna (24), Odhan (26), Bawanikhera (36) and Malaut showed DNA amplification with primers-1 and 2. Five isolates *viz.* Badhra (37), Pathrala (44), Talwandi (47), Abohar (55) and Jogiwala (65) showed amplification with primers-1 and 3. Four isolates, Hansi (14), Kalanwali (27), Mandi dabwali (32) and Sriganganagar (60) showed DNA amplification with primers-2 & 3.

DNA amplification shown with all three primers were observed in 37 isolates from different places including CCS HAU Hisar (1) , Chaudharywas (2), Aryanagar (4), Barwala (6), Unklana (7), Agroha (9), Bodha (10), Fatehabad (15), Hamjapur (16), Basin (17), Bhattukalan (18), Ding (19), Ratia (20), Kawalgarh (21), Tohana (22), Sirsa (25), Ellenabad (29), Abubshehar (33), Dudhiawali (34), Bhiwani (35), Narwana (40), Danoda Kalan (410, Unchana (420, Bathinda (43), Fazilka (49), Ganjuana (50), Kuikhera (51), Nihalkhera (52), Chuvadiawali (53), Panniwala (54), Pakki Tibbi (57), Sadhuwali (61), Sadul sahar (62), Lagarh Jatan (63), Mahiyanwali (64) and Radawali (68).

Four of the isolates from different places namely Modakhera (11), Balsamand (13), Jeevan (31) and Mahiyanwali (66) did not show any band with any of three primers suggesting no CLCuV presence (Table 4.37).

CHAPTER-V

DISCUSSION

Being an important cash crop cotton persist its position with significant values for economy of country. Cotton shares considerable per cent in country's GDP and is considered as one most demanding crop. Many pathogens attack cotton plant and induce diseases that cause severe losses in cotton production. Among the various diseases, cotton leaf curl disease (CLCuD) caused by cotton leaf curl virus (CLCuV) has become a serious threat for cotton production in cotton growing areas of North India. Presently disease has become a nuisance to the farmers as it not only affect the quality but the yield of cotton also get reduced. Since its introduction into South East Asia including India, the disease has become a big challenges for researchers to develop resistance against the virus in India. The disease has already established in northern states of India including Punjab, Haryana and Rajasthan. However, timely introduction of resistant varieties had put some control over it but not for long. Whereas, at the same time breakdown of resistance has put the major issue of sustainability of resistant cultivars However, further molecular studies on virus can help better to understand the nature of CLCuV strains those prevailed in different areas and locations of the country. In the present study epidemiological, agronomical, biochemical and molecular studies were carried out and results of study are discussed here under the following section:

5.1 Symptomatology

The study on symptomatology provided information on CLCuD identification under field conditions. Plants infected with CLCuV showed typical symptoms of vein thickening, curling of leaves and enation appeared under severe infection as previous confirmed in various studies (Briddon *et al.*, 2001 and Qazi *et al*., 2007). In the present study typical symptoms of CLCuD were observed on the infected plants of HS-6 and RCH 134 BG-II cultivars. Infected plants showed vein thickening, upward curling of leaves and enation on the underside of leaves revealed that disease showed the progression. With the progression of disease, under severe conditions flowering was also reduced and plants showed stunted growth. CLCuD was confirmed with the typical similar symptoms of the disease studied by various researchers in the past studies (Qazi *et al.*, 2007 and Farooq *et al.*, 2011). According to Monga *et al*. (2011), under severe infection the plants show reduction in seed cotton yield.

5.2 Epidemiological studies

5.2.1 Role of epidemiological factors on the development of CLCuD

5.2.1.1 Percent disease incidence (PDI) and disease progression

Study on epidemiology of disease can provide the information on the incidence and progression of disease. The disease triangle is completed by the presence of its factors and among them favourable environment is most important. The study of epidemiological factors was conducted to understand the role of these factors in the disease incidence and disease progression. Time of appearance of disease played important role in the severity and progression of disease. In the present study, the disease incidence was observed on 21st June during 2013 which resulted in to early disease progress. Whereas, disease appeared bit late (30th June) in 2014 which resulted in to delayed progress of disease. The plants suffering at seedling stage resulted into early stress conditions and have shown more disease incidence. Results revealed that disease severity reached to 72.55% and 77.05% on HS-6 and RCH 134 BG-II respectively during 2013. Whereas, during 2014 disease severity was comparatively low 57.10% and 57.30% in HS-6 and RCH 134 BG-II respectively. The results are in confirmation with Akhtar *et al.* (2004) as they also studied the role of time of infection of CLCuV and development of disease in infected plants. In the study they revealed that more incidence of disease of disease was observed on cotton plants which were infected at early seedling stage as compared to the late infection. Disease incidence favoured by the environmental factors at early crop stage probably affected the vector (white fly) activity therefore, the early incidence in 2013 was observed. Bink (1975) documented that rainfall before the seedling stage affected the white fly population and as a result disease incidence occurred at seedling stage.

Similarly, AUDPC and AIR of HS-6 and RCH 134 BG-II during 2013 showed earlier increase as compared to 2014. The disease progression was observed to be high during particular period of time in both the cultivars. The Apparent infection rate showed that rate of infection was higher from 27th to 29th weeks of the year for the two cultivars HS-6 (0.00 to 0.23) and RCH 134 BG-II (0.00 to 0.26) which revealed very fast disease progression during early seedling stage of the crop. The early infection of the virus have resulted in to early progress of the disease after infection. The results are in conformity with Meena *et al.* (2011) who studied importance of AUDPC for the progression of disease *Alternaria blight* under field condition on mustard crop. Their study revealed that the speed of progression of disease was determined by apparent infection rate and it explained the particular period of time of faster or slower progression of the disease.

5.1.4 Correlation of CLCuD with weather variables

Correlation studies of CLCuD with meteorological parameters *viz*. T_{max}, T_{min}, $RH_{morning}$, $RH_{evening}$, BSS and RF revealed that two of the weather variables T_{max}, and $RH_{morning}$ have shown significant correlation with the progression of the disease. The weather variable T_{max} have shown negative correlation, whereas, $RH_{morning}$ have shown positive correlation with the disease. Prediction equations calculated for CLCuD also indicated role of temperature and relative humidity in the epidemiology of the disease. Zafar *et al.*

(1997) observed that at high temperature virus efficiency was increased ten folds and CLCuV could be transmitted by single whitefly. The results from the study also revealed that in 2014 disease incidence was low and the regression equation showed lower per cent contribution of temperature (minimum). Monga *et al.* (2004) calculated the prediction equations for the appearance of CLCuD for 2002 and 2003. Their study revealed that maximum temperature between 35-42 °C, minimum temperature between 26-29 °C and maximum relative humidity 71-95% in the month of July favored the disease development. In present study, from stepwise regression equation the prediction equation calculated showed high R^2 value for relative humidity (morning) and temperature (maximum). Singh *et al.* (2010) also tabulated prediction equation for CLCuD based on weekly weather parameters and observed the role of temperature and humidity along with rainfall which played major role in the development of disease. The reduction in yield with the progression of disease was reported from the present study and is in with agreement with earlier studies which documented that increase in disease per cent significantly reduced the crop yield (Iqbal *et al.*, 2011 and Hussain *et al.*, 2015).

4.1.6 Whitefly population and disease incidence

Being the exclusive vector of CLCuV, study on the population build up of white fly (*B. tabaci*) was of equal importance. Therefore, role of white fly in the incidence and progression of CLCuD was observed during the season. The present study revealed that the white fly population showed very low fluctuations during 2013. Whereas, during 2014 the white fly population showed fluctuations during the crop growth period. With a downfall in white fly population, a sudden increase in number was observed within a short period (about seven days) during 2014. White fly population was increased to 92.9 and 94.8 on HS-6 and RCH 134 BG-II, respectively between 10^{th} to 12^{th} weeks after sowing in 2013. Whereas, in 2014, the population showed sudden increase at 15^{th} week after sowing. The whitefly number was increased from 93.9 to 276.3 on HS-6 and 101.8 to 293.7 on RCH 134 BG-II within a week. With the occurrence of rain, the change in weather was observed before the white fly population was increased suddenly. Bink (1975) also observed that occurrence of rainfall a week before promoted the white fly population increase. Study revealed that the overall number of whitefly during 2013 was very less as compared to the 2014. Whereas, the disease severity was high during 2013 as compared to 2014. Present study indicated that increase in white fly population have not shown the corresponding change in disease progress. Previous studies by researchers also supported the fact that the whitefly population has no correlation with incidence or progress of CLCuD under field condition. A single whitefly can successfully transmit this disease (Yassin and El-Nur, 1970). However, the same variety was not affected similarly in all the areas or within an area. The plant to plant difference in exhibiting the virus symptoms appears to be of environmental effect (Singh *et al.*, 2010). Bink (1975) also supported that the increase in white fly number before seedling stage provided more opportunity for the incidence and spread of disease. It was

already clear that the meteorological or weather parameters plays major role in multiplication of vector white fly under natural environment and also affects the epidemiology of disease significantly. CLCuD In the present study meteorological factors played important role in the epidemiology of CLCuD. In the present study, correlation study of weather parameters with the incidence of CLCuD showed significant role of maximum and minimum temperature in association with morning RH, whereas, evening RH showed less significance.

The combination of weather parameters such as Temperature and RH for a particular period creates conducive conditions for the development of the disease. The alteration in temperature and humidity significantly affects the activity of insect pests that ultimately affects disease progress (Hussain *et al.*, 2015). However, the viral diseases which are vectored exclusively might have additional concern of life cycle of its vector. Population of white fly has significant effect of weather parameters on its population buildup (Iqbal *et al.*, 2012). The egg laying capacity (fecundity) has been found influenced with the temperature and RH in the field. As a result of change in temperature and RH, the microclimate of the crop also remained to be changed under the crop canopy. Insect vectors feeding on infected plants have been reported to differ in growth rates, longevity, and fecundity compared with those vectors feeding on uninfected plants (Kennedy 1951; Baker 1960), however, some vectors preferentially colonize infected plants (Castle *et al.* 1993). Virus infection can increase plant suitability for insect vector survival and reproduction by improving the nutritional quality of the plant (Kennedy 1951; Baker 1960). However, increased survival may lead to virus proliferation in the field (McElhany *et al.* 1995). Therefore, the present study showed the role of weather factors in the incidence and development of CLCuD. The fluctuation in whitefly number did not show fluctuation in rate of increase of the disease, as the secondary inoculum was supposed to be achieved already to spread enough the disease with available whitefly in the field.

Prediction equation for CLCuD also indicated role of temperature and relative humidity in the epidemiology of the disease. The reduction in yield with progress of disease grade has been reported from the present study and is in with agreement with earlier studies of disease percent and effect on crop yield (Iqbal *et al.*, 2007). 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). The diseased plant shows physiological changes and hence ultimately yield of plant is compromised. In the present study role of various meteorological or weather parameters in white fly

population buildup was observed and early occurrence of white fly affected the epidemiology of disease significantly. Meteorological factors played important role in the secondary spread of the disease (Singh *et al.*, 2010 and Tiwari *et al.*, 2013).

5.2 Agronomic practices

Use of agronomic practice have been significant to manage the plant diseases. Effect of sowing time and plant spacing on the incidence of disease has been mentioned in various studies already (Beniwal *et al.*, 2006; Iqbal *et al.*, 2011 and Hussain *et al.*, 2015). However, the variations in results were observed in studies and it indicated the role of environment and crop factors on incidence of disease. In the present study early sown (1st May) showed least incidence of CLCuD, followed by timely sown (15th May) and late sown (1st June) crop. Disease incidence reached to 100% in late sown crop whereas, in early the disease remained below 50% and at timely sown it reached 75%. Whereas, difference of plant spacing did not show remarkable effect on difference disease incidence sown at three different time. Similar findings, were observed by Beniwal *et al.* (2006) in cotton and effect of time of sowing was significant over plant spacing in cotton crop. Early sown crop might have shown disease escape and crop attained the proper growth before the vector and disease appeared in the field. During the period of early sowing the epidemiological condition might not favourable for the progression of disease therefore, the disease incidence delayed and showed slower progression. Whereas, late sown crop prevailed favourable conditions for the progress of disease during the early plant stage i.e. early seedling stage. Hence, the plants showed severe infection at their early stage. The results are in agreement of previous studies which revealed that disease escape played major role in control of disease (Iqbal *et al.*, 2011). Iqbal and Khan (2010) also 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.

5.3 Biochemical Analysis

Plants have been known to produce a large number of secondary metabolites such as alkaloids, terpenoids, polyphenols, quinines and combined structures of these groups. Secondary metabolites are not directly essential for basic photosynthesis or respiratory metabolism in plants (Theis and Lerdau, 2003), although they play a role in defense against herbivores, microbes, viruses or competing plants to attract pollinating or seed dispersing animals. They are important for the plant survival and reproductive fitness and also protect plants from physical stresses like ultraviolet light and heat (Yazaki, 2006).

Effect of various biochemical parameters on susceptibility and resistance of host has been documented in details in various crops already. Plants shows various systemic and induced defense mechanisms against various pathogen through single or multiple biochemical pathways. Resistant plants produce certain inhibitory compounds in response to virulent pathogen which a susceptible plant could not. Therefore, in

addition to study the nature of pathogen, it is of dire importance to study biochemical compounds of resistant and susceptible plants.

5.3.1 Phenol

Among all the biochemical components of different hosts, phenols stand out as most important component in imparting resistance to several plant diseases. High concentration causes an instant lethal action by a general tanning effect while, low concentration causes gradual effect on the cellular constituent of the parasite. If the concentration does not occur in toxic level, the inhibition will be obviously slow. Besides, the pathogen readily detoxifies low concentrations of the toxicants rather than high concentrations (Dasgupta, 1988). In the present study, phenol content was increased with the age of plant up to 90 days. Highest phenol content 0.71 mg/g & 0.70 mg/g on dry weight basis was present in resistant cultivars Bunty & H-1236 at 90 DAS respectively, whereas, in HS-6 and RCH 134 BG-II it was 0.25 mg/g. Plants with highest disease grade of HS-6 and RCH 134 BG-II showed lowest amount of phenol at any particular stage of growth of plant. The results are in agreement with Ajmal *et al.* (2011) who also reported increase in phenol content in resistant plant/cultivar as compared to the susceptible plant/cultivars of cotton against cotton leaf curl virus.

Phenolic compounds may contribute to enhance the mechanical strength of host cell walls by the synthesis of lignin and suberin that are involved in the formation of physical barriers that can block the spread of pathogens (Ngadze *et al.* 2012; Singh *et al.* 2014).The variation in phenol content in different crop plants may be attributed to the unique characters of the phenolics compounds. Phenol molecule is constructed with lipophilic and hydrophilic proteins. Such molecule will be able to orient itself on oil water interface. This unique structure confirms versatilities to phenols. Phenols directly or indirectly interfere with several metabolic systems of an organism. The present findings infer that rapid accumulation of phenolic compounds occur in resistant host pathogen interaction than the susceptible host.

5.3.2 Sugar

The infection by some pathogens brings changes in respiratory pathway and photosynthesis which are the vital processes taking place inside the plant leading to wide fluctuation in sugar (Klement and Goodman, 1967; Jayapal and Mahadevan, 1968 and Patil *et al.*, 2010). Generally, high levels of total sugars, reducing sugars and non-reducing sugars in the host plants are stated to be responsible for disease resistance. Difference in sugar level between resistant and susceptible genotypes was due to inherent character of the genotypes. Sugars acts as precursor for synthesis of phenolics, phytoalexins, lignin and cellulose which play an important role in defense mechanism of plants against invading pathogens.

In the present investigation, total sugar of resistant and susceptible plants did not show any specific trend of increase or decrease in level in cotton leaves. However, plants with lower disease grade in susceptible

cultivars showed very high values of total and reducing sugar. RCH 134 BG-II plants with disease grade-0 showed highest sugar content followed by resistant cultivar Bunty (2.0 mg/g). Least amount of sugar was observed in leaves with highest disease grade-6 of susceptible cultivar HS-6 at 60 DAS, whereas, at 90DAS highest graded leaves showed higher values of sugar. The results are in agreement with Ashfaque *et al.* (2014) who also observed that disease has no significant role on foliar sugar. In their study for resistance against urdbean leaf crinkle virus in blackgram non-significant relation of sugar (total and reducing) was observed. Therefore, the present investigation can be supported with previous studies for the role of sugar content in disease resistance.

5.3.3 Gossypol

Cotton (*Gossypium hirsutum* L.) produces a number of toxic terpenoid aldehyde (TA) com- pounds contained in epidermal glands that help protect the plant from pests and diseases. One of these compounds is gossypol. Gossypol content of was also increased up to 90 DAS in all the cultivars however, no specific trend with diseased or healthy plants was observed. Heinstein *et al.* (1979) have reviewed studies of the biosynthesis of gossypol. They concluded that cis-cis farnesyl pyrophosphate (FPP) is a precursor of gossypol, and that the prenyltransferase enzyme complex (Widmaier *et al.*, 1980)

The gossypol content was highest (0.75 mg/g) in RCH 134 BG-II from plants with disease grade-4, followed by Bunty (0.74 mg/g), RCH 134 BG-II and HS-6 (0.72 mg/g). Lowest value of gossypol was observed in plants with disease grade-6 (0.47 mg/g & 0.53 mg/g respectively in HS-6 & RCH 134 BG-II.

Toxicity of gossypol to the insect pests, nematode and fungi have been already documented in cotton (Bell 1986). In some study negative correlation of biotic stress and gossypol content was observed in cotton (Hanny, 1980). Concentrations of 0.05 to 0.1 percent of rutin added to 0.1 percent gossypol greatly increased toxicity to bollworms, indicating a synergistic interaction between flavonoids and terpenoids in natural resistance to insects (Lukefahr and Martin, 1966). No correlation of gossypol with resistance to pests was observed by Namazov *et al.* (2008) also. Therefore, in the present study the concrete information on role of gossypol on resistance might not be documented from the results obtained.

5.3.4 Protein

The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis, particularly during incompatible reaction. Quantitatively the total protein synthesis is much enhanced in the tissues around the infected tissues. Involvement of protein components in plant diseases resistance has been documented in many plant pathogenic interactions (Tornero *et al.*2002 and Carvalho *et al.*2006).

Protein content of resistant cultivar Bunty was highest (26.3 mg/g) at 90 DAS, followed by H-1236 (25.1 mg/g). The susceptible cultivars with lower disease grade showed more protein content as compared to plants with higher disease index. The results are in agreement with the findings of Beniwal *et al.*, 2006, Acharya and Singh (2008) and Siddique *et al.* (2014) who observed the role of protein in resistance of

cotton plant against CLCuD. According to Agrios (2005), infected plants show a high protein content, which could be due to both the activation of the host defense mechanisms and the pathogen attack mechanisms. Therefore, the role of protein in disease resistance has been a matter of study always. The present study however, indicated that resistant cultivar might have shown stronger defense mechanism against CLCuV and more protein production as compared to the susceptible cultivars.

5.3.5 Tannin

Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins. Tannins are considered to be the most important secondary plant compound involved in plant defense against insects and disease (Swain, 1979). Howell *et al.* (1976) and Sadykov (1972) observed that flavonol (catechins and condensed tannins) concentrations decrease gradually in leaves with ageing that are synthesized earlier in leaf development.

In the present investigation, tannin content of susceptible cultivars (HS-6 & RCH 134 BG-II) showed trend of decrease in values with the increase in CLCuD grade index and resistant cultivars (H-1236 & Bunty) showed higher values also. However, overall tannin content was decreased after 60 days of sowing. Plant with lower disease grade showed higher values of tannin % as compared to plants with more disease grade. HS-6 plant with grade-0 showed 0.76 mg/g whereas, grade-6 plant showed 0.47 mg/g at 60 DAS. Similarly, RCH 134 BG-II plants showed 0.93 mg/g tannin at grade-0 and 0.61 mg/g at disease grade-6. The results are in agreement with the findings of Beniwal *et al.* (2006) and Acharya and Singh (2008) who also observed the role of tannin in resistance against CLCuV. In earlier studies against fungal pathogens, role of tannins have been shown and inhibited the growth of Cladosporium caryigenum, the causal organism of pecan scab, *in vitro* (Laird, 1990) and to be strongly correlated with grain mold resistance in sorghum (Menkir, 1996). Also, the concentration of tanningreatly increases 24 h after inoculation with *Rhizoctonia*, with greater post-infection increases coming from older plants (Hunter, 1974). Therefore, in the present study also the role of tannin in resistance in cotton plant might be important however, exact mechanism is still to be understood for CLCuV.

5.3.6 Chlorphyll (a & b)

Chlorophyll, carotenoids (CAR) and phenolics (PHE) are commonly studied metabolites of plant kingdom (Ajmal *et al.*, 2011). Secondary metabolites synthesis including chlorophyll, increase under stress condition and is believed to protect the cellular structure oxidative damage (Buchanan *et al.*, 2000). In the present study chlorophyll content was increased with increase in grade and age of the plant. Plants with higher disease grade showed more Chlorophyll (a & b) as compared the plants with lower disease grade and of healthy plants. Chlorophyll content of older plants was higher as compared the chlorphyll content at younger stage in resistant and susceptible plants together. The results are in agreements with Ashfaq *et al.* (2014), as they also observed higher chlorophyll content in susceptible plants of blackgram

infected with urdbean leaf crinkle virus as compared to the healthy plants. Role of chlorophyll with susceptibility of plant was studied in the past. As, magnesium is present in chlorophyll and plays very important role in synthesis of chlorophyll and consequently in photosynthesis and carbohydrate metabolism (Devlin and Witham 1983; Kandhasamy *et al.*, 2010). Increase in magnesium and zinc was observed in susceptible plants with increase in photosynthesis and more chlorophyll content (Reddy *et al.*, 2005). The results are also in agreement with Brar and Rataul (1990) who reported significant increment in total chlorophyll contents in ULCV-infected old leaves of urdbean.

5.3.7 Peroxidase (PO)

Flavanols and their oxidation products formed with peroxidase act as bactericides (Venere, 1980), fungicides (Howell et at., 1976; Hunter, 1978), enzyme denaturants (Hunter, 1974 and 1978) and antisporulants (Howell *et al.*, 1976). Hunter (1978) showed that low levels of catechin stimulated pectinase production by a strain of *Rhizoctonia solani* highly virulent to cotton, whereas only inhibitory effects occurred with moderately virulent strains. As peroxidase is assumed to be one of the enzymes responding and providing fast defense against plant pathogens (Sulman *et al.* 2001).

The present study exhibited that PO activity was more in resistant cultivars H-1236 & Bunty. At 30 DAS peroxidase activity was 1.8 to 34.5 min -1/g of leaf in H-1236 and 1.7 to 34.8 min -1/g in Bunty. Similarly, in healthy leaves of susceptible cultivar HS-6 it increased from 1.7 to 34.0 min -1/g and in healthy leaves of RCH 134 BG-II it was 2.1 to 33.1 min -1/g. Results of study are in agreement with Siddique *et al.* (2014) who also observed higher activity in healthy plants of susceptible cultivar as compared to the diseased plants. In present study resistant plants showed more activity of PO enzyme. The findings are supported with the previous study on cotton leaf curl virus (Siddique *et al.*, 2014). Similar studies have been reported on other viral diseases on various crops such as tomato & bellpepper (Madhusudan *et al.* 2009), cucumber (Riedle-Bauer, 2000), tobacco (Kiraly *et al.* 2002), blackgram (Ashfaque *et al.*, 2010), tomato (Dieng *et al.* 2011) and a numberof resistant interactions involving several plant pathosystems might be there.

5.3.8 Polyphenol (PPO)

PPO is important in the initial stage of plant defense in response to the membrane damage that results into release of phenols such as chlorogenic acid. PPO catalyzes the oxidation of phenolics to free radicals that after reaction may create unfavorable environment for pathogen development (Jockusch 1966 and Mohamed *et al.* 2012).

The trend of activity of PPO in the present study indicated that more PPO activity was shown in resistant plants. Whereas, decrease in activity of PPO was observed with the increase in disease grade. PPO activity was highest in resistant cultivar Bunty at 30 DAS (10.2 to 18.9 min -1/g) followed by H-1236 which showed activity from 10.1 to 18.3 min -1/g of fresh leaves. Leaves of grade-0 from RCH 134 BG-

II also showed high activity (10.2 to 17.4 min -1/g) followed by HS-6 (from 9.5 to 16.8 min -1/g). Similar findings were observed by Siddique *et al.* (2014) for PPO activity in resistant and susceptible cultivars of cotton tested against cotton leaf curl virus. Previous studies on different crops also suggested that PPO is induced in the plants in response to the infection of any pathogen (Constabel *et al.* 2000; Stewart *et al.* 2001; Vanitha*et al.* 2009). The exact mechanism of enzyme activity is a matter of study however, the possibility of some common mechanism can not be overlooked in case of cotton leaf curl virus infection in the present investigation.

5.4 Molecular analysis

Molecular studies of any of pathogen or host can help to understand the differences in pathotypes at genetic level. The field observations and studies that showed variations in response of a host to different types of pathogen or different isolates of a pathogen reveals some genetic association of host and pathogen (Gupta *et al.*, 2010a). Molecular level studies of a pathogen or organism can unveil the basic differences and help researchers to understand the nature of the pathogen/organism. In the present study the molecular diversity of various isolates of CLCuV were studied. Out of 145 diseased samples isolates of cotton leaf curl disease collected from Haryana, Punjab and Rajasthan during 2013, sixty eight (68) showed good quality DNA isolation and bands of DNA were observed on the agarose gel. These isolates showed variable results after amplified with coat protein specific markers. Four isolates were observed to show amplification only with Primer-1 (CtLCV #33), seven isolates were amplified with Primer-2 (CtLCV #45) and three showed amplification with Primer-3 (CtLCV #55) only. Among all, five isolates have shown amplification with Primer-1 & 2, five different isolates were amplified with Primer-1 & 3 and four isolates showed amplification with Primer-2 & 3. Out of 68 total isolates, 36 isolates have shown DNA amplification with all three coat protein specific primers (Primer-1, 2 & 3). There were four isolates that did not show amplification with any of three primers used in the study. These primers amplified different domains of the CLCuV coat protein gene. Viruses from different samples might have sequence modifications in different regions of the stretch of gene. The viruses demonstrated diverse amplification patterns suggesting diverse alterations in coat protein.

Variations in response to these primers indicated that the probability of diversity at molecular level might have been presented already. However, further confirmatory tests can reveals the actual differences at very minor level. The response of isolates to the amplification with primers indicated that most of isolates share common coat protein DNA sequences, whereas, in case of few isolates different markers with different sequences showed variations in amplification of the isolates. Tiendrebeogo *et al.*, (2010) observed variations in amplifications of okra yellow vein mosaic virus strains. They concluded that, the samples showed variation in amplification due to CP ORF specific amplification that was present in some of the isolates even showing diseased symptoms. In the present study the possibility of specific

ORF amplification might have been involved in response to the amplification of CLCuV with CP specific primers. This also did not confine the possibility that there are lack of diversity among isolates of CLCuV. Seven species of Begomovirus have been identified so far in India which showed variations in size of DNA, functions and location on genome (Iqbal *et al.*, 2014). Researchers have observed some difficulties in isolation of CLCuV isolates and study them at molecular level. Some factors play role in hindering the characterization of virus DNA. The major constraints in study includes lack of proper rearing of whitefly to study mode of transmission under *in vitro* conditions, difficulty of purification of virus particle due to their presence in phloem tissues and abundance of secondary metabolites and polysachharides in cotton that interfere with virus isolation (Brown, 1997). The acquisition capacity of whitefly can not be overlooked as it affects the transmission and severity of the disease (Gupta *et al.*, 2010b). Response of various cultivars also varies towards the virus infection due to some physiological, molecular and biochemical differences at host or vector level (Tiwari *et al.*, 2013). Biotypes identified in different areas of the world suggesting that *B. tabaci* may be a species complex undergoing evolutionary change. These biotypes may exhibit differences in viruses transmitted and transmission efficiencies, rates of development, endosymbionts, host utilization, and physiological host damage (Oliveira *et al.* 2001). The response of unknown cotton cultivars grown in the particular field or area showed variable response to the virus and therefore, the possibility of variations in host-pathogen-vector interaction could also affect the study on diversity at molecular level. However, Kuldeep (2013) studied the diversity of various isolated of CLCuV collected from different places of Haryana, Punjab and Rajasthan and the study revealed the diversity among isolates of cotton leaf curl virus at molecular level. His study exhibited the presence of four variants of CLCuV from the diseased samples. However, any geographical pattern was not observed as per of his studies. In the present study the possibility of diversity was observed at initial genetic level among various isolates of CLCuV collected from cotton belt of Haryana, Punjab and Rajasthan where various species of CLCuV are prevalent already. Earlier studies on variability showed that genome of CLCuBuV was a recombinant and indicated the possibility of existence of new race of Begomovirus (Ashraf *et al.* 2013). The major constraint of present study would be the less numbers of molecular markers used. Use of molecular markers specific to resistance against CLCuV could add to further studies. Therefore, molecular markers associated with cotton leaf curl disease resistance can enhance the selection efficiency in breeding programmes and would provide more selective protocols for the study of CLCuV (Farooq *et al.*, 2011).

CHAPTER-VI

SUMMARY AND CONCLUSION

Cotton is the principal cash crop and has significant contribution into Indian GDP. It is dual purpose crop as it provides fibre as well as edible oil. In India it is grown in north, south and central zones. The fibre productivity has crossed 467 kg/ha. Crop suffers from a number of diseases and insect-pests throughout its growing period. Among many limiting factors of low productivity, cotton leaf curl disease (CLCuD) is the most crucial in north western India. Present study was conducted to study the symptomatology of the disease, the role of environmental factors in the epidemiology of cotton leaf curl virus (CLCuV). The role of date of sowing and spacing on the incidence of CLCuD. The possible role of plant biochemical to the response of disease was studied through biochemical analysis of resistant and susceptible cultivars of cotton. There have been reports of new strains development of CLCuD therefore, CLCuV isolates collected from various cotton growing area of north India were brought under molecular analysis to study the variations among them. The salient features of the present investigation are summarized below:

Study on symptomatology revealed that cotton plants infected with CLCuV produced typical symptoms of CLCuD under natural epiphytotic conditions. The infected plants showed a series of symptoms from small vein thickening, big vein thickening, leaf curling (upward/downward), appearance of enation at underside of leaves and under severe conditions stunted growth of plants was observed. The symptoms were confirmatory to CLCuD and were grouped as per the scale suggested by AICCP.

Present studies revealed that disease appeared early during 2013 as compared to 2014 which resulted into more disease severity in 2013 as compared to 2014. However, the progression of disease was faster during 2014 as compared to 2013 and disease reached to its maximum at the same stage of plant. Therefore, study revealed that favourable environment during particular period of the year promoted the disease progression.

From the studies it was observed that white fly started to appear early during 2013 as compared to 2014. The studies revealed that early vector appearance resulted into early disease appearance during 2013 as compared to 2014. During 2013, white fly number increased continuously, whereas, downfall and sudden rise in white fly population during 2014 was observed. Overall white fly population during 2014 was very high as compared to 2013. The fluctuation and increase in population of white fly during 2014 did not show any correlation with the progression of disease.

The result of the present study indicated the weather variable played important role in the progression of disease during both the years. Regression analysis was also calculated for the disease

progression in relation to various weather variables. Cumulative regression explained that contribution of weather variables in the disease progression was about 88% during 2013 and during 2014 it was about 70%. From the stepwise regression analysis it was revealed that Temperature (maximum) and RH (morning) played important role in the progression of disease. Results also revealed that contribution Temperature (minimum) was low during the year 2013. Whereas, during 2014 Temperature (minimum) contributed more. Correlation analysis for disease progression in relation to weather variables revealed that Temperature (maximum) and Temperature (minimum) showed negative correlation with disease development. Whereas, RH (morning) showed positive correlation. Whereas, RH (evening), BSS and RF did not show correlation with CLCuD in cotton.

Effect of time of sowing and plant spacing was also studied to observe the incidence and severity of CLCuD during the season. In early sown crop, the disease appeared at later stages of crop as compared to timely and late sown crops. Crop sown at 1st May showed late appearance of CLCuD and overall least incidence and severity of disease was observed. Crop sown at 15th May showed incidence at earlier stages of crop as compared to the early sown crop and exhibited more disease severity. Whereas, late sown crop was infected at very early plant stage and plants were severely infected at their early stage. The disease incidence and severity was very high on plants from late sown crop.

Effect of disease severity on various plant characters and seed cotton yield was also observed. Overall yield of early sown crop was highest whereas, yield in late sown crop was significantly lowest during both the year. Rate of disease progress was slower in early sown crop as compared to late sown cotton crop which showed increasing rate of disease progress during both the seasons (2013-2014). The effect of spacing on incidence of CLCuD was not significant on the crop sown at same date. Whereas, more disease incidence was observed late sown crop.

The role of various plant biochemical of cotton plant in the disease resistance against CLCuV was also studied. For the various biochemical constituents *viz.* phenol, sugar (total and reducing), tannin, gossypol, protein, chlorophyll (a and b), peroxidase and polyphenol oxidase activity were observed among diseased and healthy plants. Two susceptible cultivars HS-6 (hybrid) and RCH 134 BG-II (Bt) and two resistant cultivars H-1236 (hybrid) and Bunty (Bt) were used for biochemical studies.

The results revealed that the resistant cultivars H-1236 and Bunty showed very high phenol content whereas, diseased plant of susceptible cultivars HS-6 and RCH 134 BG-II exhibited very low phenol from plants with highest disease grade (grade-6). Therefore, the study revealed that phenol has its role in the resistance against CLCuD. Plants showed reduction in phenol content near maturity at 120 days after sowing.

The results of the study revealed that sugar (total and reducing) content of susceptible and resistant cultivars of cotton did not show any relation with the resistance against CLCuD in hybrids and Bt cotton.

Perusal of data revealed that gossypol content was increased with the age of plant for all the disease grades. No specific trend or relation of gossypol was observed with the susceptibility or resistance in plants. However, overall gossypol content showed increasing trend with the age up to 90 DAS and thereafter, gossypol was decreased.

Results of the present study revealed that higher tannin content was observed in healthy and resistant (grade-0) plants as compared to the diseased plants of susceptible cultivars (hybrid and Bt). The tannin content showed increasing trend up to 60DAS in all the cultivars and thereafter it showed the decreasing trend. Therefore, it can be concluded that tannin content contribute in resistance during early stages of plant.

Results from the present study revealed that the protein content was changed with the plant reaction against the disease. High protein content in resistant cultivar was present as compared to diseased plants. Therefore, role of protein content was observed in inciting resistance against CLCuD.

In the biochemical studies conducted for chlorophyll revealed that chlorophyll (a) content was significantly higher in highly diseased plants as compared to healthy plants/resistant plants. Whereas, Chlorophyll (b) did not show the significant difference in diseased and healthy plants. The overall content of chlrophyll-a was higher than that of chlorophyll-b in healthy and diseased plants.

Enzyme activity of diseased and healthy plants of susceptible and resistant cultivars of cotton was also studied. Perusal of data revealed that peroxidase activity was higher in healthy (0 grade) plants of susceptible and resistant cultivars. The activity was increased in all types of healthy or diseased plants. However, the activity of enzyme was faster in healthy and resistant plants as compared to the diseased plants which showed slower activity. Similarly, polyphenol oxidase activity was more in healthy and resistant plants as compared to the diseased plants. However, the trend in PO activity observed was similar in all grades of plant.

Molecular studies were also carried out for the samples collected from various cotton growing areas of Haryana, Punjab and Rajasthan. The CLCuV strains were subjected to amplify with coat protein specific primers for CLCuV.

The result revealed that primers used in the study exhibited differences in DNA bands for different strains of CLCuV. Among the 68 samples used for the amplification, 36 strains showed presence of bands in all three primers (CtLCV #33, 45 and 55), only four of the strains showed amplification with Primer-1 (CtLCV #33), seven strains showed amplification with Primer-2 (CtLCV #45), three strains were amplified only with Primer-3 (CtLCV #55) and four strains did not show any

amplification with any of three primers. The study revealed that monomorphic results did not show variability in amplified DNA of various isolates. However, the presence or absence of band with particular isolate indicated complex system of analysis of host-virus-vector relation. The possibility of diversity/variations among the CLCuV strains can not be overlooked, however, the availability of less numbers of primers might be one of the constraints of showing diversity at molecular level.

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Annexure-I

Weekly weather data of year 2013

Std. Met. Week	DAS	Period	Temp. °C		RH %		Bright sun hours	Rain fall (mm)
			Max.	Min.	M	E		
22	7	28.5.13 - 3.6.13	41.6	24.1	56.9	25.6	10.5	0.0
23	14	4.6.13 - 10.6.13	43.4	28.5	60.3	31.1	8.2	0.0
24	21	11.6.13- 17.6.13	35.5	25.0	81.1	73.4	4.0	97.3
25	28	18.6.13- 24.6.13	37.9	27.1	67.4	33.1	9.1	0.0
26	35	25.6.13 - 1.7.13	39.6	28.4	70.6	39.9	6.8	0.0
27	42	2.7.13 - 8.7.13	38.7	27.5	78.1	55.0	6.9	51.5
28	49	9.7.13 - 15.7.13	35.7	26.5	82.6	60.9	7.8	65.7
29	56	16.7.13 - 22.7.13	36.1	26.4	84.4	67.1	5.5	30.5
30	63	23.7.13 - 29.7.13	35.3	27.1	85.4	59.0	7.6	0.0
31	70	30.7.13 - 5.8.13	34.6	26.9	86.9	67.1	7.0	69.0
32	77	6.8.13- 12.8.13	32.7	25.9	92.0	76.0	4.5	76.5
33	84	13.8.13- 19.8.13	31.7	25.5	96.0	81.0	7.1	154.2
34	91	20.8.13- 26.8.13	33.6	26.1	91.4	68.7	5.9	0.0
35	98	27.8.13 - 2.9.13	35.3	25.3	77.7	55.3	9.9	0.0
36	105	3.9.13- 9.9.13	35.2	23.7	77.6	46.7	10.0	0.0
37	112	10.9.13 -16.9.13	36.5	24.6	83.6	44.0	9.1	0.0
38	119	17.9.13 - 23.9.13	32.8	22.4	88.1	58.1	7.3	114.5
39	126	24.9.13- 30.9.13	32.7	24.5	90.1	70.1	6.3	25.9
40	133	1.10.13- 7.10.13	31.3	23.6	89.1	62.7	6.9	4.0
41	140	8.10.13 -14.10.13	32.0	23.6	91.6	58.9	5.8	2.5

Annexure-II

Weekly weather data of year 2014

Std. Met. Week	DAS	Period	Temp. ^{0}C		RH %		Bright sun hours	Rain fall (mm)
			Max.	Min.	M	E		
22	7	28.5.14 - 3.6.14	41.2	23.7	61	26	9.3	9.0
23	14	4.6.14 - 10.6.14	44.5	26.2	48	19	10.1	0.0
24	21	11.6.14- 17.6.14	41.2	26.6	61	40	9.0	37.5
25	28	18.6.14- 24.6.14	40.5	27.3	66	38	7.3	10.6
26	35	25.6.14 - 1.7.14	38.0	26.3	69	44	6.5	14.5
27	42	2.7.14 - 8.7.14	38.0	27.6	65	44	5.2	0.0
28	49	9.7.14 - 15.7.14	38.5	28.2	64	43	4.8	0.0
29	56	16.7.14 - 22.7.14	38.5	27.8	67	50	4.7	7.6
30	63	23.7.14 - 29.7.14	37.6	27.7	70	52	3.8	9.1
31	70	30.7.14 - 5.8.14	35.6	26.9	91	64	6.5	33.2
32	77	6.8.14- 12.8.14	35.2	26.8	83	55	5.3	0.0
33	84	13.8.14- 19.8.14	36.1	26.5	78	48	8.6	0.0
34	91	20.8.14- 26.8.14	38.5	25.8	70	37	10.4	0.0
35	98	27.8.14 - 2.9.14	35.3	25.5	84	61	6.3	1.0
36	105	3.9.14- 9.9.14	33.0	25.5	92	67	5.4	76.7
37	112	10.9.14 -16.9.14	34.4	24.7	89	59	7.0	4.8
38	119	17.9.14 - 23.9.14	36.2	22.9	77	39	10.0	0.0
39	126	24.9.14- 30.9.14	36.0	21.6	74	38	9.9	0.0
40	133	1.10.14- 7.10.14	37.0	21.7	76	32	9.5	0.0
41	140	8.10.14 -14.10.14	34.6	18.9	80	39	9.0	20.3

ABSTRACT

Title of Thesis	:	Studies on Epidemiology and Molecular Diversity of Cotton Leaf Curl Virus (CLCuV) in Cotton (*Gossypium hirsutum* L.)
Name of Degree holder	:	**PRASHANT KUMAR CHOHAN**
Admission Number	:	2012A44D
Title of Degree	:	Doctor of Philosophy
Name and address of Major Advisor	:	Dr. Naresh Mehta, Professor, Dept. of Plant Pathology CCS HAU, Hisar, Haryana-125 004
Degree Awarding University	:	Chaudhary Charan Singh Haryana Agricultural University Hisar -125 004
Year of award of Degree	:	2016
Major Subject	:	Plant Pathology
Total number of Pages in Thesis	:	95 + XII
Number of words in the Abstract	:	Approx. 402

Key words: Cotton, CLCuD, epidemiology, agronomy, biochemical parameters and molecular diversity

Present investigation comprised two cultivars HS-6 and RCH 134 BG-II susceptible to CLCuV used for study on epidemiology, effect of date of sowing and spacing on the incidence of cotton leaf curl disease (CLCuD). Biochemical studies included phenol, sugar (total and reducing), tannin, protein, gossypol, chlorophyll-a & b, peroxidase and pholyphenol oxidase analysis using standard biochemical methods. Four cotton cultivars HS-6 & RCH 134 BG-II (susceptible) and H 1236 & Bunty (resistant) were used in the study. For molecular study, cotton leaves showing CLCuD symptoms were collected from different cotton growing locations of Haryana (Sirsa, Fatehabad, Hisar, Bhiwani and Jind), Punjab (Abohar, Bathinda and Fazilka) and Rajasthan (Hanumangarh and Shriganganagar). Results exhibited that disease was appeared early in season during 2013 whereas late in 2014, however, fluctuation in white fly population during 2014 did not affect CLCuD progress. Negative correlation of CLCuD was observed with T-max and T-min, whereas, RH (morning) showed positive correlation. In early sown crop, the disease appeared at later stages of crop as compared to timely and late sown crops. Overall yield of early sown crop was highest whereas, yield in late sown crop was significantly reduced in two year of study. The effect of spacing on incidence of CLCuD was not significant. Biochemical studies revealed that phenol content was high in resistant plants whereas, low in diseased plants. Tannin content was high in resistant plants as compared to diseased plants. However, gossypol and sugar (total and reducing sugar) content changes did not show any relation with CLCuD infection in plants. Protein content was increased with the age of plant. Chlorophyll (a & b) content was higher in diseased plants as compared to healthy/resistant plants. Peroxidase and Polyphenol oxidase activity was higher in healthy (0 grade) and resistant plants in comparison to the diseased plants. Molecular study revealed that primers used in molecular level study showed differences in DNA amplification. Among the 68 samples, 36 showed DNA presence in all three primers, 4 showed in Primer-1, 7 in Primer-2, 3 in primer-3 and 4 samples did not show amplification in any of the primers. Five samples showed amplification with primer-1 & 2, 5 in primer-2 & 3 and 4 samples showed presence in primer-1 & 3 only. Monomorphic results observed did not show variability in amplified DNA of various isolates. However, the presence or absence of band with particular isolate indicated complex system of analysis of host-virus-vector relation.

MAJOR ADVISOR **SIGNATURE OF STUDENT**

HEAD OF THE DEPARTMENT

CURRICULUM VITAE

1.	**Name**	**:**	**Prashant Kumar Chohan**
2.	**Date of Birth**	**:**	**November 04, 1980**
3.	**Place of Birth**	**:**	**Karnal**
4.	**Mother's Name**	**:**	**Smt. (late) Mithlesh Chauhan**
5.	**Father's Name**	**:**	**Sh. Biram Pal Singh**
6.	**Permanent Address**	**:**	**VPO – Gonder, Teh. – Nissing**
			Dist. – Karnal (Haryana) Pin- 132001
7.	**Mobile**	**:**	**+91 9996904994**
8.	**E-mail**	**:**	pchauhan.k@gmail.com

Academic qualifications:

Degree	University/Board	Year of passing	Percentage of marks	Subjects
Ph.D.	CCS HAU, Hisar	2016	74.45%	Plant Pathology
M.Sc.	CCS HAU, Hisar	2005	72.50 %	Plant Pathology
B.Sc (Hons.) Agriculture	CCS HAU, Hisar	2003	68.00%	All Agricultural subjects
10+2	HBSE, Bhiwani	1998	54.0 %	English,Hindi, Physics Chemistry, Biology
Matriculation	HBSE, Bhiwani	1996	55.4 %	English, Hindi, Maths, Science,Social Science

Achievements **:** Nil

List of publications (Ph.D.) **:** National conferences (2)

Chohan, P.K., Jagdish Beniwal, N. Mehta, R.S. Sangwan and V.K. Sikka. 2015. Effect of time of sowing and spacing on incidence of cotton leaf curl disease. Annual Meeting of Inidan Phytopathological Society and Indian Society of Mycologoy and Plant Pathology (North Zone) & Natioan Symposium on "Holistic Approach for Plant Health Management", September 28-29, YS Parmar University of Horticulture and Forestry, Nauni, HP. p125.

Chohan, P.K., Jagdish Beniwal, R.S. Sangwan and N.K. Mehta. 2016. Role of biochemical constituents in resistance against cotton leaf curl disease (CLCuD) in cotton (*Gossypium hirsutum* L.). National Conference on Genetic Diversity and Therapeutic Potential of Natural Products, September 17, MDU, Rohtak, Haryana. p97.

[Signature of Student]

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