

Review Article

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An Overview on Cotton Leaf Curl Disease: An Emerging Potential Threat to Cotton

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ABSTRACT

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Cotton leaf curl disease (CLCuD) caused by cotton leaf curl virus (CLCuV), a geminivirus is one of the most destructive disease of cotton. The losses due to cotton leaf curl disease vary from 10-90% across the cotton growing areas of Punjab, Haryana and Rajasthan. Cotton leaf curl virus belongs to family Geminiviridae, genus begomovirus and consists of monopartite genome with circular, single stranded DNA. Typical symptoms of CLCuD, includes thickening of veins and upward or downward curling of leaves, vein darkening and enations. CLCuV subsist on alternate hosts like Brinjal, Cucurbits, *Convolvulus arvensis*, *Rumex dentatus*, Water melon, Cow pea and Lilly plants from where it is transmitted exclusively by vector whitefly (*Bemisia tabaci*) to the healthy cotton crop. CLCuD lowers fiber quality traits like ginning out turn, fiber length, fiber uniformity index, fiber fineness, fiber bundle strength, and maturity ratio also deteriorated because of change in composition of major fiber components including cellulose, protein, wax and pectin. The obliteration of alternate host and restraining of vector whitefly will be advantageous in control of CLCuD.

Introduction

Cotton has an eminent position in Indian agricultural, industrial and economic development. In India main cotton growing belt is north-western region including Punjab, Haryana and Rajasthan. In India, it occupies an area of about 118.81 lakh ha with total production 352 lakh bales and average yield is 503 kg/ ha (Anonymous, 2016). Several constraints are responsible for decreased production as well as productivity of cotton in India among them cotton leaf curl disease has

emerged as one of the most important one. Among four cotton cultivated species, only two viz., *G. hirsutum* L. and *G. arboreum* L. are grown in Haryana. *G. hirsutum* L. is susceptible whereas, *G. arboreum* is resistant to Cotton leaf curl disease (CLCuD). The disease is caused by whitefly transmitted Geminivirus which was first noticed in Nigeria on *Gossypium peruvianum* and *G. vitifolia* (Farquharson, 1912). In India the disease was first reported on *G. barbadense* at

Indian Agricultural Research Institute, New Delhi in 1989, followed by American cotton (*G. hirsutum*) in Sriganganagar district of Rajasthan in 1993 (Ajmera, 1994)

Cotton leaf curl disease (CLCuD) caused by single stranded circular Geminivirus consisting of DNA-A and two satellites, exclusively transmitted by the insect vector whitefly (*Bemisia tabaci*). It belongs to genus Begomovirus, and is transmitted by its exclusive vector whitefly (*Bemisia tabaci*) in circulative and persistent manner. Apart from cotton, the virus can infect several collateral and alternate hosts that act as source of inoculum for its spread from one season to the other by its vector *i.e.* whitefly. However, symptoms of the disease have not been properly defined on weeds. A number of weeds as well as other hosts harbour this virus in the off season and serve as the main source of primary inoculum for the development of disease on cotton. Identification of weeds which carrying CLCuV inoculum and its timely detection can help in rouging of weed plants to prevent further spread of the virus.

Cotton Leaf Curl Virus

A notorious group of the virus family *Geminiviridae*, causes major threat to cotton crop and a well-known threat is Cotton leaf curl disease (CLCuD) caused by Cotton leaf curl virus (CLCuV), transmitted in persistent manner by whitefly *i.e.* *Bemisia tabaci* (Brown *et al.*, 1995). CLCuV is most closely related to 'Old World' viruses such as Indian cassava mosaic and tomato yellow leaf curl. Seven diverse species of begomoviruses have been found which is associated with the CLCuD in the Indian subcontinent specifically Cotton leaf curl Multan virus (CLCuMV), Cotton leaf curl Rajasthan virus (CLCuRV), Cotton leaf curl Alabad virus (CLCuAV), Cotton leaf curl Kokhran virus (CLCuKV) and Papaya leaf curl virus

(PaLCuV), Cotton leaf curl Bangalore virus (CLCuBV) associated with alphasatellite and betasatellite molecules (Mansoor *et al.*, 2003b; Briddon 2003) and Cotton leaf curl Burewala virus (CLCuBuV) (Amrao *et al.*, 2010).

PCR-mediated amplification showed the presence of a begomovirus named *Cotton Leaf Curl Virus* (CLCuV) in CLCuD-infected cotton. A smaller (approx. 1350 nucleotides) single stranded DNA molecule (termed DNA-1) was shown to be associated with CLCuD (Mansoor *et al.*, 1999). This molecule capable of self-replication in plant cells but requiring CLCuV for spread in plants as well as in insect transmission was shown to play no part in inducing symptoms. This DNA 1 shows high level of similarity to nanovirus components which encode the replication associated protein (RAP). Attempts to identify a second genomic component (DNA-Beta) were not successful earlier, suggesting that CLCuV is a monopartite begomovirus.

However, full length clones of CLCuV, although infectious, were unable to induce the symptoms typical of CLCuD in cotton and other host plants. This indicated that it requires one other factor to induce symptoms of the disease in plants (Briddon *et al.*, 2001). The genome component designated DNA-A encodes viral functions required for replication and also plays an essential role in insect transmission. The second component encodes the products involved in movement within and between the cells in host tissues (Briddon *et al.*, 2001).

However, a small number of begomoviruses such as CLCuV, *Tomato yellow leaf curl virus* (TYLCV), *Ageratum yellow vein virus* (AYVV) etc. have a monopartite genome. In monopartite begomoviruses, these genes are present in DNA-A. Recently a single stranded DNA molecule approximately 1350

nucleotides in length has been isolated and identified which when coinoculated with the begomovirus to cotton, induces symptoms typical of CLCuD, including vein swelling, vein darkening, leaf curling and enations (Briddon *et al.*, 2001; Briddon *et al.*, 2003; Mansoor *et al.*, 2003; Kirthi *et al.*, 2004; Radhakrishnan *et al.*, 2004). A distinct strain of cotton leaf curl Burewala virus (CLCuBV) also was identified in C49 isolate collected from CLCuD symptomatic cotton plant in Layyah district, Punjab. The newly identified strain of CLCuBV lacks an intact transcriptional activator protein (Shuja *et al.*, 2014).

Genome of CLCuV monopartite

Genome of CLCuV is monopartite genome, but in some species of CLCuV small circular single-stranded DNA satellites are associated with some monopartite begomoviruses, including cotton leaf curl Multan virus (CLCuMV), tomato yellow leaf curl China virus (TYLCCV), and they have been shown to be important for the induction of disease symptoms (Briddon *et al.*, 2001). DNA β satellites are approximately 1350 bp and require a helper virus for replication and encapsidation (Mansoor *et al.*, 2003; Briddon & Stanley, 2006). Analysis of DNA β sequences has revealed a conserved organization consisting of a single complementary-sense gene (β C1) an adenine-rich region and a satellite-conserved region that carries sequence similarity to the *Tomato leaf curl virus* (ToLCV) satellite-DNA (Briddon *et al.*, 2003). The DNA β associated with cotton leaf curl disease (CLCuD) encodes a single protein called fiCl which is a pathogenicity determinant (Saeed *et al.*, 2005). A new betasatellite DNA is reported that associated with the Cotton leaf curl Burewala virus infecting tomato plants in India influence on symptoms and viral accumulation (Kumar *et al.*, 2013).

Symptomatology of CLCuD

Cotton leaf curl disease symptoms were first reported in Nigeria in *G. Barbadense* cotton (Farquharson, 1912). In 1924, similar symptoms were reported to be widespread in the Sudan cotton crop (Golding, 1930), Symptoms of cotton leaf curl virus disease may vary from variety to variety but a specific virus produces the same symptoms in different hosts (Kirkpatrick, 1931; Bedford *et al.*, 1994).

Tarr (1951) reported spirally twisted petioles, fruiting branches and tall stems and elongated internodes in *G. barbadense*. Most varieties in Africa infected by CLCuGV exhibit dwarfing, overall stunting, reduced boll number and boll weight. Two types of vein thickening are produced by CLCuD *i.e.*, main and small vein thickening. 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 extended and resulting in more or less continuous reticulation of veins (Nour and Nour, 1964; Watkins, 1981). Begomoviruses 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.*, 1993).

The typical symptoms of the disease include upward or downward leaf curling, dark green veins, vein thickening and enation that appeared in bead-shaped, small fine leaf like structures on the under surface of the leaves (Bink, 1975; Briddon and Markham, 2000). The severely affected plants have bushy appearance with dark green colour, short

internodes without flowers and bolls (Chauhan, 2004).

Alternate hosts of Cotton Leaf Curl Virus

Nour and Nour (1964) observed leaf curl symptoms on *Corchorus fascicularis*, *Phyllanthus niruri*, *Clitoria sp*, *Phaseolus vulgaris* and *Petunia*. The important alternate hosts reported from Sudan are species of *Gossypium*, *Hibiscus*, *Althaea*, *Sida*, *Malva*, *Malvaviscus*, *Abutilon*, *Lavatera* and possibly *Urena* (EI and Salih, 1970). In Pakistan under field conditions CLCuV symptoms were observed on alternate hosts like Brinjal, Cucurbits (“Tinda”, “Kali tori”), *Convolvulus arvensis*, *Rumex dentatus*, Water melon, Cow pea and Lilly plants (Anonymous, 1993). Alternate hosts play an important role in causing this virus to spread over cotton crop. They provide inoculum to the vector for transmitting the same in cotton. There are many ornamental plants and common weeds which have been found infected with whitefly-transmitted geminiviruses (Mansoor *et al.*, 1998; Mansoor *et al.*, 1999). Singh *et al.*, (1994) reported appearance of the CLCuD on the *Sidasps*, *Abutilon indicum*, *Hibiscus rosa-sinensis*, and *Althea roses* on the basis of visual symptoms. In *Phaseolus vulgaris*, pepper, tomato and tobacco, transmission studies and ELISA showed the presence of CLCuV (Nateshan *et al.*, 1996).

Cotton leaf curl virus disease CLCuD spread widely from the basal inoculums that are present in the off season in the form of weeds and other hosts (Monga *et al.*, 2001). Radhakrishnan *et al.*, (2001) conducted transmission studies on Okra, Hollyhock, Tomato, *Physalis floridana*, *Nicotiana benthamiana* and French bean to detect the presence of cotton leaf curl virus. DNA-A probe hybridization conducted on Hollyhock, *Sida ageratum*, China rose to detect the

presence of CLCuV (Sharma and Rishi, 2003). *Tribulus terrestris* and *Cucumis* spp. showed the presence of DNA-A and Beta DNA of CLCuV in their genome (Sivalingam *et al.*, 2004). *Corchorus acutangularis*, *Melilotus indica*, *Ageratum conyzoides* were detected CLCuV positive on the basis of DNA-A & DNA beta probe hybridization (Radhakrishnan *et al.*, 2004). PCR using CP primer showed the presence of CLCuV genome in Tomato, Tobacco, Zinnia, Jungli pudina, Chilli, China rose, Okra, *Sida alba*, *Sida* sp., *Achyranthes* sp. and *Clerodendron* sp. (Kang *et al.*, 2004, Monga *et al.*, 2005). *Convolvulus arvensis*, *Capsicum spp.*, *Parthenium sps*, *Solanum nigrum*, *Digitaria arvensis*, *Lantana camara*, *Achyranthes aspera*, *Chenopodium album*, *Spinacea spp.*, *Xanthium strumarium* were also reported as host of CLCuD coat protein gene amplification (Monga *et al.*, 2011).

PCR for detection and diagnosis of viruses

In general, PCR is very useful in detection and diagnosis of viruses, viroids and other plant pathogens. Geminiviruses are well studied due to its ease of detection and identification by PCR, as they replicate via double stranded, circular DNA intermediate, replicative form (Stanley, 1991). Which serve as a template for amplification by PCR. The genome constitute of number of regions which are highly conserved between viruses and hence can be used to design degenerate PCR primers (Rojas *et al.*, 1993; Tan *et al.*, 1995; Muniyappa *et al.*, 2000). Degenerate oligonucleotide primers, designated for amplification of an approximately 500 bp fragment of DNA-A of five well characterized whitefly transmitted geminiviruses were used in the polymerase chain reaction (PCR), to detect known or putative geminiviruses mostly infect seven plant species that are originally obtained from Europe, India, Africa and America (Deng *et*

al., 1994). The sequence of a pair of degenerate primers suitable for the PCR mediated amplification of near full length genomes of Geminiviruses that infect dicotyledonous plants has been described (Bridon and Markham, 1994). The products of these amplifications were useful not only as diagnostic probes but also for the determination of DNA sequence and single cutting restriction endonuclease sites prior to cloning of viruses. Degenerate primers designed to amplify DNA-A of whitefly transmitted geminiviruses were used in PCR for amplification of viral DNA (Mansoor *et al.*, 1998). A product of expected size was 1.4 were obtained from all these samples that confirmed the infection of whitefly transmitted Geminiviruses. A set of primers designed based on the conserved sequence of coat protein region were used for the detection of DNA-A in cotton leaf curl virus (CLCuV) infected weeds and cotton plants using PCR technique (Monga *et al.*, 2005). Similarly, primers Beta 1 and 2 designed to amplify DNA- β were used (Monga *et al.*, 2005). The cotton leaf curl virus specific primer precisely detected the virus in infected cotton as well as in symptom less plants by amplification of 771 bp coat protein gene (Chakrabarty *et al.*, 2005).

Losses and appearance of CLCuD

Cotton leaf curl disease is the most devastating natural calamity that reduces the quality and production of the cotton (Viveki *et al.*, 2014). Disease is reported to cause huge loss in various regions for instance, considerable seed cotton yield reduction in Rajasthan (32.9 to 50.3%), Punjab (10.5 to 92.2%) and Haryana (39.4 to 81.4%) states of north India has also been reported (Narula *et al.*, 1999; Monga *et al.*, 2001). Maximum seed cotton yield (q/ha) was recorded in Bt hybrid SP 7007 BG (27.8) followed by Bioseed 6488 BGII (26.9) and SP 7007 BGII

(26.5). Percent maximum loss in seed cotton yield was observed in hybrid Jai BG (54.1) followed by MRC 7361 BG (48.8) and Solar 76 BG (46.8) (Monga *et al.*, 2011).

Appropriate sowing time preferably mid-April to mid-May results in decrease of disease incidence (Ghazanfar *et al.*, 2007) as compared to delay in sowing from mid-May to June. Amid various management factors to boost up per acre yield of cotton, apposite genotype selection, optimal sowing time and higher plant density toning the ecological conditions of the region is the most important (Ali *et al.*, 2004; Nadeem *et al.*, 2010). Iqbal and Khan (2010) reported that increased plant spacing in the case of early sowing and decreased plant spacing under late sown conditions is effective in management of CLCuD. They also concluded that CLCuD infestation reached its maximum after 105 days of sowing and in case of late sown crop *i.e.* 15 June or later infestation becomes severe after 45 days of sowing. They recommended 15 cm plant spacing in order to manage CLCuD in the case of planting later than 15th of June. CLCuD exhibited a negative and significant association with seed cotton yield, GOT, no of bolls (Saeed *et al.*, 2014 and Farooq *et al.*, 2013). CLCuV severely hampered the crop performance by delayed planting of cotton from 15th April; while increased the chances of disease incidence. It concluded that early sowing reduces the problem of CLCuV and enhanced cotton productivity (Ali *et al.*, 2014). Due to CLCuD incidence fiber quality traits like ginning out turn, fiber length, fiber uniformity index, fiber fineness, fiber bundle strength, maturity ratio also deteriorated because of change in composition of major fiber components including cellulose, protein, wax and pectin (Farooq *et al.*, 2014). Maximum CLCuV incidence was recorded within 30-45 days in late planting as compared to 105 days of early planted cotton. Delay in sowing

cotton crop reduced seed cotton yield progressively (Hussain *et al.*, 2015).

Cotton leaf curl disease (CLCuD) is one of the key threat to the cotton crop, leading to about 10 – 90% loss. Control of vector whitefly by applying relevant cultural, mechanical and biological approaches has been found effective to manage CLCuV. Numerous studies has been conducted for the detection of alternate/collateral hosts of CLCuV and screening of resistant germplasm against CLCuV, but not fully understood. Therefore, there is dire need to identify the primary source of inoculum i.e. collateral and alternate weeds/hosts, assessment of losses and source of resistance to CLCuD for its mitigation and management.

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