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CLCuD: Study of different aspects in relation to upland cotton (*Gossypium hirsutum* L.)

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Abstract
Cotton leaf curl disease (CLCuD) is a severe and potential threat for cotton production in India. Cotton leaf curl disease is caused by cotton leaf curl virus (CLCuV) which is a single stranded circular Geminivirus that consists of DNA-A and two satellites. This virus belongs to the genus *Begomovirus* and transmitted by the insect vector whitefly (*Bamicia tabaci*) in the circulative and persistent manner that causes drastic reduction in the yield, growth and quality parameters of the cotton crop. Cotton leaf curl disease can be escaped or more appropriately the impact of cotton leaf curl disease can be minimized by modifying the different management practices in such a way so that susceptible stages of the crop does not coincide with the environmental conditions that favours CLCuD. These different management strategies can be adopted for this which includes; promotion for the cultivation of desi species *i.e. G. arboreum* L. and *G. herbaceum* L., identification of the resistant sources and making attempts to pyramid resistant genes, the resistant genes against CLCuD may be transferred or introgressed into upland cotton from *Gossypium herbaceum* L., *Gossypium arboreum* L. and also from the related wild species for which biotechnological tools can be used, destruction of the infected plants especially after harvesting, early sowing to escape pest and disease infestation, destruction of off-season weeds and clean cultivation during the season to minimize sources of virus inoculums. Adoption of strategies like early sowing to minimize/ avoid the infestation of CLCuD having very less per cent disease incidence and whitefly population as compared to late sown crop, balanced use of fertilizer and eradication of host plants from field after harvesting may be helpful to minimize the loss in production & productivity and helpful for enhancement of quality.

Keywords: *Begomovirus*, cotton, CLCUD, upland cotton, whitefly

Introduction
Cotton is the most important cash crop of India that belongs to the genus *Gossypium* and family Malvaceae. Cotton is also known as White Gold and it is the principal source of foreign exchange earnings in many countries. It is the most important *kharif* cash crop of North India. Among the various factors responsible for its low production and productivity during the last one and a half decade, cotton leaf curl disease (CLCuD) has been found to be one of the major limiting factor. The disease has assumed serious proportions in the most potential irrigated cotton belt of North India. Cotton leaf curl virus disease is caused by cotton leaf curl virus (Sattar *et al.,* 2013) [40]. Whitefly (*Bemisia tabaci*) is the vector of this disease (Rajagopalan *et al.,* 2012; Briddon, 2015) [37, 13] which transmits *Geminivirus*. It is the most devastating pathogen of cotton, which is responsible for causing huge economic yield losses (Humza *et al.*, 2016) [20]. *Geminivirus* interacts in a persistent, circulative manner with its vector *i.e.* whitefly and is commonly known as CLCuD-associated *Begomovirus* (CBVs) which belongs to the genus *Begomovirus* and family Geminiviridae. Previously, it was thought that only CLCuV was responsible for causing cotton leaf curl disease, but recent investigations have suggested that there is the involvement of a virus complex in causing the disease. Recent advances that are made in development of new resistant varieties/ hybrids, use of and growing the resistant germplasm to combat with CLCuD, epidemiological studies including development of disease maps, detection of new weed hosts, breakdown of resistance due to development of new viral recombinants along with future management strategies such as adoption of different planting space and timings will be discussed in this review article.

Cotton leaf curl disease (CLCuD)
Cotton leaf curl virus disease which is a serious threat to cotton crop and transmitted by Whitefly (*Bemisia tabaci*) is the major problem in cotton cultivation (Sharma *et al.,* 2006) [42]. Cotton leaf curl virus (CLCuV) is most closely related to 'Old World' viruses such as Indian cassava mosaic and Tomato yellow leaf curl and Papaya leaf curl virus (Mansoor *et al.,* 2003b; Briddon, 2003) [40, 14].

CLCuD is caused by a pathogen complex of a virus and a DNA beta satellite (DNA- b) molecule (Tahir *et al.,* 2011) [46]. There are about seven such species of virus, all of which belongs to the genus *Begomovirus* and DNA-b satellites are associated with CLCuD in these regions (Ahuja *et al.,* 2007; Briddon 2003; Mansoor *et al.,* 2003b; Mansoor *et al.,* 2006; Azhar *et al.,* 2010) [2, 14, 30, 31, 12].

A small number of *Begomovirus* such as CLCuV, Ageratum yellow vein virus (AYVV) have a monopartite genome. In monopartite *Begomovirus*, these genes are present in DNA-A. 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) [15]. Recently a single stranded DNA molecule approximately 1350 nucleotides in length has been isolated and identified which when co-inoculated 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; Kirthi *et al.,* 2004; Radhakrishnan *et al.,* 2004) [15, 14, 27, 36]. A distinct strain of cotton leaf curl Burewala virus (CLCuBV) was also identified in C-49 isolate collected from CLCuD symptomatic cotton plant in Layyah district, Punjab. Shuja *et al.* (2014) [43] reported that the newly identified strain of CLCuBV lacks an intact transcriptional activator protein.

Some techniques or strategies for screening/ control/ management of CLCuD

Conventional methods and Inter-specific Hybridization

Conventional breeding methods have certain limitations because of the sudden changes in climatic conditions and availability of limited resources. The available germplasm of upland cotton is susceptible to CLCuD as reported (Anonymous, 2011b) [10]. Cultivated species of desi/ diploid cotton *i.e. Gossypium herbaceum* (A1) and *Gossypium arboreum* (A2) are resistant to this disease as reported (Anonymous, 2011a) [9]. From genus *Gossypium* eight wild diploid species are found resistant to CLCuD as reported (Anonymous, 2011a) [9].

In order to make inter-specific crosses successful *i.e.* between tetraploids (*Gossypium hirsutum* L.) and diploid (*Gossypium arboreum* L.); Gibberalic acid was used to overcome shedding of inter-specifically crossed bolls (Mofidabadi, 2009) [34]. Ahmad *et al.* (2011) [1] reported that boll retention in cross of *Gossypium arboreum* with *Gossypium hirsutum* or in reciprocal cross was very low but F_1 and BC_1 population of this cross were resistant to CLCuD. An autotetraploid of *G. arboreum* L. was created and were manually hybridized with allotetraploid *G. hirsutum* under field conditions and the BC_2 population showed resistance to CLCuD, this was also reported by Ahmad *et al.* (2011) [1]. These findings indicates that use of conventional breeding methods to transfer desirable traits from diploid species are feasible to some extent and efforts should continue to transfer the gene resistant to CLCuD from diploid species mentioned above in upland cotton. Also with the advancements in biotechnological methods now it is easy to combat cotton leaf curl virus by cloning certain viruses and develop controlling strategies (Farooq *et al.,* 2011) [17].

Induce mutation

Cultivation of the resistant cotton genotypes is the most effective, safe and economic method of reducing yield losses caused due to CLCuD. Akhtar *et al.* (2005) [4] studied and reported that PIM-76-8/5 is a new CLCuD-resistant line developed through the use of induced mutation. Most of the mutants that were resistant or moderately resistant under BLGT inoculation were found immune or highly resistant under natural conditions. None of the test mutants was found susceptible or highly susceptible under field conditions reported by Akhtar et a*l.* (2000) [3].

Grafting method

Root stock, the cotton genotype to be tested against CLCuD and scion consisted of the susceptible source of disease inoculums to transmit the disease in stock plants. Later on presence of virus was confirmed visually and after that ELISA test was carried out for further confirmation (Farooq *et al.,* 2011) [17]. Many scientists (Ali M., 1997; Akhtar *et al.,* 2004; Akhtar *et al.,* 2010; Shah *et al.,* 2004 and Mansoor *et al.,* 2003a) [7, 5, 6, 41, 29] used this method. Three procedures of grafting are mostly applied by the researchers which include bottle graft, top cleft and wedge graft.

Inoculation of CLCuD through grafting

Nazeer *et al.* (2014) [35] carried out a study in which a petiole and rootstock from CIM-496 were used to transfer virus inoculum into healthy plants. Two grafting techniques *i.e.* approach and petiole grafting were employed to confirm the resistance against CLCuD in BC_1 to BC_3 plants. For the technique of grafting such as approach grafting, the resistant plants of the BC_1, BC_2, and BC_3 progenies were used as scions whereas virus-susceptible plants of *G. hirsutum* L. were used as stock. For another method of grafting *i.e.* petiole grafting, young petioles from CLCuD-infected plants were selected and inserted into the test plants. Two infected petioles were also grafted onto the same plant to introduce additional virus inoculum. The assessment of CLCuD through grafting showed that the BC_1 to BC_3 progenies were highly resistant to this disease. Nazeer *et al.* (2014) [35] also successfully demonstrated the possibility of introgressing CLCuD resistance genes from *G. arboreum* to *G. hirsutum.*

Impact of plant spacing and planting time (Early sowing)

It was reported in a study 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. So screening of candidate genotypes or segregating material for CLCuD infestation tolerance should be planted in the 1st or 2nd week of July. This method is economically most feasible to screen germplasm, segregating population and candidate varieties against CLCuD tolerance.

The impact of plant spacing and planting time on yield components of cotton and incidence of CLCuD was studied and a significant interaction of plant spacing and planting time for seed cotton yield, its component traits and CLCuD incidence was observed. Higher seed cotton yield in early planting with high plant spacing and maximum yield with narrow plant spacing in late planting was observed. The incidence of disease and also the intensity increased in late sowing of the crop (Iqbal *et al.,* 2008; Iqbal and Khan, 2010; Tanveer and Mirza, 1996; James *et al.,* 2004) [22, 23, 47, 24]. Iqbal *et al.* (2008) [22] reported and suggested that cotton genotypes those fell prey to severe incidence of CLCuD can be managed to withstand the damage by increasing the population of plants and application of the nitrogen fertilizer to achieve optimum seed cotton yield.

Inheritance pattern / genetical aspect of CLCuD

Knight (1948) [28] reported that CLCuD is under control of a major gene. Siddiq (1970) [24] suggested that a major dominant gene is involved in controlling resistance of CLCuD along with minor (modifier genes). Studies and findings of Ali (1999) [8], Rehman *et al.* (2002) [39] and Haider (2002) [19] suggested that CLCuD is controlled by single gene with dominant effects. Iqbal *et al.* (2003) [21] carried out a study and reported the involvement of two dominant genes and also they behaved as dominant epistasis in controlling resistance to CLCuD. Rahman *et al.* (2005) [38] reported the involvement of three genes in *Gossypium hirsutum* resistance to CLCuD *i.e.* two for resistance (R_1 CLCuD hir and R_2 CLCuD hir) and a third suppressor of resistance (SCLCuD hir).Quantitative inheritance with predominance of additive gene effects for CLCuD resistance was revealed by Khan *et al.* (2007) [26].

It was reported in a study that genetic tolerance can be intensified by gene pyramiding; two new cotton genotypes MNH 886 and IUB 222 have developed through gene pyramiding which are highly tolerant to CLCuD (Anonymous, 2011b) [10]. Genetic pyramiding involved stacking of naturally occurring alleles of tolerant genes into a single elite genotype in multiple crossing attempts. Monogenic tolerance did not prove successful for longer period of time and is always at risk in the current world wide viral threat. Godara *et al*. (2016) [18] studied the inheritance of cotton leaf curl virus disease (CLCuD) in four crosses that involved resistant and susceptible parents to this disease and depicted the duplicate dominant (15 resistant: 1 susceptible) effect for inheritance of cotton leaf curl virus disease in upland cotton. Sonika and Sangwan R.S. (2017) [45] revealed by their study that the inheritance of cotton leaf curl virus disease indicated the complementary type of gene interaction (9:7).

The successful exploitation of the source of resistance also requires information on the genetic control in order to breed a resistant variety. To achieve this goal, an understanding of mode of inheritance of cotton leaf curl virus along with other yield attributing traits is necessary for proper choice of breeding procedures. The information on these aspects may help in selection and adoption of breeding approaches suitable for improving yield and attributing characters. Hence, the knowledge of inheritance pattern /genetics of cotton leaf curl virus may be helpful in designing/ adopting breeding procedure for the development and cultivation of resistant genotypes to combat with CLCuD.

Biotechnological tools & techniques to combat with CLCuD

Biotechnology refers to the application of various biological organisms and processes for production of useful substances or effects in the field of agriculture. Molecular markers associated with cotton leaf curl virus disease resistance can enhance the selection efficiency in breeding programmes (Farooq *et al.,* 2011) [17]. By using markers, selection for resistance would be easy without being infecting the plants with the pathogen, thereby reducing the chance of pathogen to escape into new environment (Aslam *et al.,* 2000) [11]. Aslam *et al.* (2000) [11] found three DNA marker loci that were linked with each other and had association with cotton leaf curl virus by evaluating a subset of F_2 plants using selective genotyping with RFLPs.

RNA interference is cutting edge technology which can be effectively utilized in the development of resistance against CLCuD (Kasschau and Carrington, 1998; Waterhouse, 2001; Mikhail *et al.*, 2003) [25, 48]. Post transcriptional gene silencing is found to be useful for RNA viruses while geminiviruses are effectively controlled by transcriptional as well as post transcriptional gene silencing. Mette *et al.* (2000) [32] advocated the effectiveness of transcriptional gene silencing against Mung Been Yellow Mosaic Virus. This gives an improvisation for the effective utilization of RNAi in the control of CLCuD.

Conclusion

CLCuD is a serious threat for cotton production so this disease should be tackled in an efficient way so that crop can be saved from its devastating effects and production and productivity of crop can be maintained. Different Management strategies can be adopted for this such as Promotion for the cultivation of desi species *i.e. Gossypium arboreum* and *Gossypium herbaceum*; Identification of the resistant sources on priority basis and attempts must be made to pyramid resistance genes, Biotechnological tools can be used for identification of resistant sources and introgression of resistant genes into upland cotton; Destruction of the infected plants especially after harvesting; Crop rotation with the crops that are not host plants for whiteflies; Early sowing to escape pest and disease infestation; Destruction of off-season weeds and clean cultivation during the season to minimize sources of virus inoculums; Development and implementation of effective strategies for whitefly management.

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