J. Cotton Res. Dev. **35**(2), 197-203 (July, 2021) **ISSN No. 0972-8619**

Inheritance pattern of resistance to cotton leaf curl disease (CLCuD) in *Gossypium hirsutum* L.

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ABSTRACT : Cotton leaf curl disease (CLCuD), caused by begomoviruses in association with satellite molecules, is the biggest threat to cotton production and productivity in north India. The line GVS-9 showed resistance to CLCuD, and it was crossed with two CLCuD susceptible genotypes CSH 3129 and F 2228, seperatly and F^1s were developed. F_1s were planted for disease reaction and reported resistant, also selfed to develop F_2s. The segregation behavior of disease reaction on F_2s showed a segregation pattern of 3 (resistant) : 1 (susceptible) ratio, thereby indicating that the dominant gene governs the CLCuD resistance. The chi-square for goodness of fit was 1.204 with p (0.05) of 0.2725 and also represented a test for 3 (resistant): 1 (susceptible) ratio, confirming that the resistance to CLCuD is governed by a single dominant gene.

Keywords: Cotton leaf curl disease, F_1s, F_2s, gene, goodness of fit

Cotton has manifold merits, it is used as fibre, lint for making clothes, food, feed, and cottonseed oil. Linters use in manufacturing of high grade paper and rayon, and also for making X-ray films. *G. hirsutum* cotton occupies the major area, *i.e.* more than 90 per cent of 12.2 million ha. (Anonymous 2020; https://pib.gov.in/PressReleasePage.aspx?PRID=1605056). In the present scenario, Cotton leaf curl disease (CLCuD) is the major threat to cotton production and productivity in north India. CLCuD is caused by begomovirus (family: *Geminiviridae*) having single-stranded (ss), circular DNA genomes and associated with satellite molecules *viz.* betasatellite and alphasatellite and it is transmitted through its vector, whitefly (*Bemicia tabaci*) Brown *et al.*, 2015, Sattar *et al.*, 2017; Zerbini *et al.*, 2017). The CLCuD disease on *G. hirsutum*, was first reported during 1989 at the Indian Agricultural Research Institute, New Delhi and subsequently in 1993 at Sriganganagar district of Rajasthan state (Gupta and Kumar, 2017). In a short span of 4-5 years it covered entire northern India region of cotton cultivation and presently is the major threat to *G. hirsutum* cotton area of this region (Gupta and Kumar, 2017). Begomoviruses occurring across India (Northern India) and Pakistan have complex genetic structures and it has been observed that they are fast changing and varies from region to region. Mainly four Begomoviruses viruses *viz.*, Cotton leaf curl Rajasthan virus (CLCuRV), Cotton leaf curl Mutan virus (CLCuMuV), Cotton leaf curl Kokhran virus (CLCuKV) and Tomato leaf curl Bangalore virus-cotton (ToLCBV) are prevalent in India (Ahuja *et al.*, 2007). Another virulent, resistance breaking Cotton leaf curl Burewala virus (CLCuBuV) is predominantly prevalent in Pakistan and India during 2009-2010 (Rajagopalan *et al.*, 2012, Iqbal *et al.*, 2014 and Rahman et al., 2017). Recently the CLCuD epidemic during 2015-2016 was caused by CLCuMuV in North India (Datta *et al.*, 2017). A recent study has indicated that the beta satellite molecules of CLCuD shared 95–99 per cent nucleotide sequence identity with Cotton leaf curl Multan betasatellite (CLCuMB) while alphasatellite molecules revealed 98 per cent identity with Guar leaf curl alphasatellite (GLCuA) which is earlier reported from Pakistan (Quadir *et al.*, 2019).

Thickening of small veins on upper young leaves is the initial symptom of the CLCuD, which later induce curling of leaves (both upward and downward). Even enations are present on leaves infected with this disease. The disease is transmitted through whitefly, but no correlation has been established that whitefly is directly responsible for spread and severity of disease. However, a highly significant and positive correlation between viruliferous whitefly population and per cent diseases index of CLCuD on cotton plants was observed (Kumar *et al.*, 2019). Moreover, it was also reported that minimum temperature and sunshine hours have significant negative correlation with the incidence and progress of the CLCuD. It was also observed that humidity and rainfall have positive correlations (Bhattacharyya *et al.*, 2017).

To find out the source of resistance/ tolerance, the whole pool of germplasm available at ICAR-CICR, Nagpur was screened against CLCuD at ICAR-CICR, Regional Station, and none of the gemplasm line was observed to be resistant. Since 2005, *Bt* cotton hybrids were introduced in north zone of India and presently more than 90 per cent area of cotton crop has come under *Bt* cotton varieties and hybrids, including BG I and BG II hybrids recommended for Northern India are not having absolute resistance to this disease. There was a severe epidemic of CLCuD in north India during 2009-2010 crop season (Rajgopalan *et al.*, 2012). Even tolerant varieties/hybrids showed susceptible reaction at hot spot areas of CLCV incidence. Begomoviruses causing CLCuD overcome the resistance of cotton during 2009-2010 due to recombination of events over the years coupled with favourable environmental conditions. CLCuKoV-Bu was associated with the 2009–2010 CLCuD outbreak in Punjab and Rajasthan states of India (Monga *et al.*, 2011; Rajagopalan *et al.*, 2012). Recently, during 2015 another CLCuD epidemic caused by CLCuMuV was experienced with whitefly outbreak (Dutta *et al.*, 2017).

MATERIALS AND METHODS

Screening of GVS 8 and GVS 9 for CLCuD disease

Two CLCuD resistant *G. hirsutum* lines GVS 8 and GVS 9 were imported from USA during 2015-2016 and were planted at farm of ICAR-Central Institute for Cotton Research, Regional Station, Sirsa in the CLCuD disease screening nursery. No CLCuD incidence was observed on GVS 8 and GVS 9 genotypes, for two consecutive years. These lines were also ratooned for the next year's crop and no disease was reported on ratooned plants also.

Observations of CLCuD on each cotton plant were recorded by observing CLCuD symptoms The observations were taken at 0-6 disease ratting scale (DRS) as described in the Table 1 (Akthar *et al.*, 2010; Monga, 2014). Total numbers of plants showing leaf curl virus disease symptoms (upward/downward curling with thickened vein on underside of leaf) were counted every time during the observations and percent disease incidence was calculated as given below:

$$\text{Per cent disease incidence} = \frac{\text{Infected plants}}{\text{Total plants}} \times 100$$

Per cent disease intensity/Index (PDI) was also calculated by using the formulae:

$$\text{PDI} = \frac{\text{Sum of numerical rating}}{\text{Total number of plants x 6 (maximum severity grade)}} \times 100$$

Development of F_1 and F_2 population

During 2016-2017 crop season, GSV 9 was crossed with two genotypes susceptible to CLCuD namely, CSH 3129 and F 2228 and F1s were developed. Both F1s (GSV 9 x CSH 3129 and GVS 9 x F 2228) were planted during 2017-2018 and disease reaction was recorded on F1s and no disease was reported. The F1s were ratooned to 2018-2019 and no CLCuD disease was reported on ratooned plants also. During 2017-2018, F_1s

Table 1. Cotton leaf curl virus disease ratting scale used during the study

S. No.	Symptoms	Disease rating scale (DRS)/ symptom severity scale	Per cent disease	Disease response index
1	Complete absence of symptoms	0	0	Immune
2	Symptoms of vein thickening on few upper leaves	1	0.1 - 10	Highly Resistant
3	Symptoms of vein thickening, cupping and curling on few upper leaves	2	11 - 20	Resistant
4	25 per cent plant affected with vein thickening, cupping and curling, leaf enations	3	21- 30	Moderately Resistant
5	50 per cent plant affected with vein thickening, cupping and curling, leaf enations	4	31 - 40	Moderately Susceptible
6	75 per cent plant affected 5 with vein thickening, cupping and curling, leafy enation	5	41- 50	Susceptible
7	Plants stunted severely and complete plant affected with vein thickening, cupping and curling and leafy enation	6	>51	Highly Susceptible

of both the crosses were selfed to develop F2s. A total of 204 plants were raised in F2 for cross GVS 9 x CSH 3129 and 134 plants for GVS 9 x F 2228. Numbers of resistant and susceptible plants were counted in respective F2s. CLCuD reaction observed in segregation generations were subjected to chi-square test for goodness-of-fit.

RESULTS AND DISCUSSION

The cotton crop season in the north zone of India normally begins with sowing during mid-April to mid-May. The cotton crop sown before April attracts early CLCuD infection. The first symptom of CLCuD infection appears within 50 days of sowing among susceptible genotypes (Ahuja *et al.*, 2007). However, the progress of the disease was reported to be in increasing trend from August to October with maximum during the month of August as compared to July and September in Northern India (Monga *et al.*, 1998, Kumar *et al.*, 2019). During the early part of the cotton growing season, the maximum temperature touches to 45-46°C and there is severity of expression of CLCuD symptom. CLCuD screening is difficult as the disease occurrence depends on vector whitefly, susceptible host and favourable environment. During 2015-2016 there was epidemic of whitefly in Northern part of India, which caused favorable conditions for CLCuD occurrence and expression of symptoms and no disease symptom of CLCuD were observed on GVS 8 and GVS 9. In later years also there was good occurrence of the disease and favoured the screening of the material against CLCuD. The GVS 9 based on its consistency of being noted as resistant and F 2228 and CSH 3129 as susceptible parents for three consecutive years 2016 to 2018. There were 8 F1 plants of cross GVS-9 x CSH-3129 and none of them showed symptom of CLCuD during two consecutive years 2017 to 2018 (Table 2). Likewise, in the cross of GVS-9 x F2228, 4 F1 plants were resistant and no symptoms were observed during two consecutive years 2017 to 2018. There were 204 F2 plants in the cross of GVS-9 x CSH-3129 and out of them 163 plants were observed resistant while 41 were observed with mild to severe symptoms of CLCuD and they were treated as CLCuD susceptible. The segregation pattern of F2 in this cross was observed 3 (resistant) :1 (susceptible) thereby indicated that the gene responsible for CLCuD resistance is dominant. The chi square (S«2) value for goodness of fit was 2.614 with p (0.05) of 0.1059. In another cross of GVS-9 x F2228, there were 134 F2 plants and out of them 106 plants were observed resistant while 28 were CLCuD

Table 2. Cotton Leaf Curl Disease reaction in F_1 and F_2 population of the crosses between GVS-9 x CSH-3129 and GVS-9 X F 2228

Year	Entry	CLCuD reaction		Total	Expected Plants	S«²p Ratio (R:S)	=0.05	
		Resistant (R)	Susceptible (S)					
2016 to 2018	GVS-9	2	0	2	-	-	-	
2016-2017 to 2018	CSH 3129	0	4	4	-	-	-	
2017-2018	F_1 (GVS-9 x CSH-3129)	8	0	8	-	-	-	
2018-2019	F_1 (GVS-9 x CSH-3129)- ratooned	8	0	8	-	-	-	
2018-2019	F_2 (GVS-9 x CSH-3129)	163	41	204	3:1	2.614	0.1059	
2016-2017 to 2018	F 2228	0	4	4		-	-	
2017-2018	F_1(GVS-9 x F2228)	4	0	4		-	-	
2018-2019	F_1(GVS-9 x F2228)- ratooned	4	0	4		-	-	
2018-2019	F_2 (GVS-9 x F2228)	106	28	134	3:1	1.204	0.2725	
Pooled	**269**	**69**		**338**	**3:1**	**3.791**	**0.05153**	

susceptible as they were having CLCuD symptoms. The segregation pattern of F2 in this cross was also observed 3 (resistant) :1 (susceptible), which supported that the dominant gene governs the CLCuD resistance. The chi square (S«2) value for goodness of fit was 1.204 with p (0.05) of 0.2725. The homogeneity chi-square value was also well within the accepted limit. Therefore, the data were pooled and summed data chi-square thus represented a test for 3 (resistant) :1 (susceptible) ratio, confirming that the resistance to CLCuD is governed by single dominant gene. Earlier findings of Ali (1997), Rehman *et al.*, (2002), Haider (2002), Mahmood (2004) and Rehman *et al.*, (2005) has also suggested that CLCuD is controlled by a single gene with dominant effects.

Whereas, some workers have also reported different kinds of ratio of resistance to susceptible in segregating generations. Siddiq (1970) suggested that a major dominant gene is involved in controlling resistance of CLCuD along with minor (modifier genes). Kumar (2002) investigated the inheritance of CLCuD and reported a phenotypic ratio of 15 (resistant): 1 (susceptible). Sajjad *et al.*, (2003) reported that a single dominant gene control with modifiers. Iqbal *et al.*, (2003) reported the involvement of two dominant genes and behaved as dominant epistasis in controlling resistance to CLCuD. Rehman *et al.*, (2005) reported the involvement of three genes in *G. hirsutum* resistance to CLCuD, two for resistance (R1ClCuDhir and R2 ClCuDhir) and a third suppressor of resistance (sClCuDhir). Quantitative inheritance with predominance of additive gene effects for CLCuD resistance was also revealed by Khan *et al.*, (2007). Likewise Ahuja *et al.*, (2007) reported 4 types of segregation pattern in F2 generations, 15 (resistant) :1 (susceptible), 13 (resistant) :3 (susceptible), 9 (resistant) :7 (susceptible) and three gene control with triplicate dominant epistatsis which lacked segregation. Inheritance of cotton leaf curl virus disease (CLCuD) was studied in four crosses involving resistant and susceptible parents, six generations i.e. P1, P2, F1, F2, BC1 and BC2 were generated. Resistant reaction was dominant over susceptibility as all the plants in F1 generation were resistant in both R x S crosses and R x R cross. All the plants in F1 generation of S x S cross were susceptible. In F2 generation of both R x S crosses showed the duplicate dominant (15 resistant: 1 susceptible) effect for inheritance of cotton leaf curl virus disease in upland cotton. These results were further confirmed by observed ratio in backcross generations. The F1 s of crosses *viz.*, GCH 3 x HS 6, GCH 3 x RST 9, H 1353 x HS 6 and H 1353 x RST 9 had resistance to CLCuD, indicated that resistance is a dominant trait. The expression of

resistance in all (R x S) crosses revealed that there was no cytoplasmic inheritance for the expression of susceptibility to CLCuD. The pattern of segregation in F2 gave a good fit to 9 resistant: 7 susceptible indicated the presence of complementary type of gene action and presence of dominant alleles of both the genes controlled the resistant trait. Disease was expressed in those plants which had any one of the two or both the genes in the homozygous recessive state. Complementary type of gene interaction for CLCuD was further confirmed by a good fit of 3 resistant: 1 susceptible ratio of backcrosses with susceptible parents. During 2018 Mushtaq *et al.*, reported that resistance gene analogues (RGA) and ESTs (expressed sequence tags) expressed only in *G. arboreum* and in asymptomatic plants of *G. hirsutum*, could be useful in the study of resistance against CLCuV in addition to that RGA and ESTs did not express in Coker might be helpful in the study of CLCuV resistance. Several workers have reported from one gene to two genes hypothesis and also quantitative genes responsible for explanation of inheritance of the disease. Due to the fast rate of change of recombination events of the virus, the single or two genes become suppressed over the time. A comprehensive strategy including genotypes with broad genetic base and resistant genes from different sources may provide durable and sustainable resistance to CLCuD.

CONCLUSION

In the present study, in both the crosses of susceptible genotypes with resistant genotype GVS 9 a segregation pattern of 3 (resistant) : 1 (susceptible) was observed, which is indicating that the dominant gene is responsible for CLCuD resistance. Hence, is is proposed that dominant resistant gene needs to be transferred to elite cultivars through repeated backcrossing and for long term sustainability of resistance multiple crosses may be attempted to have diverse populations.

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Received for publication : April 17, 2021
Accepted for publication : June 21, 2021