

Genetical basis of cotton leaf curl virus disease in upland cotton (*Gossypium hirsutum* L.)

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ABSTRACT : The present investigation comprising of 6 generations (P_1 , P_2 , F_1 , F_2 , BC_1 and BC_2) of 4 crosses in cotton *viz.*, GCH 3 x HS 6, GCH 3 x RST 9, H 1353 x HS 6 and H 1353 x RST 9 was conducted to study the inheritance of cotton leaf curl virus disease (CLCuD) from *kharif* 2013 to 2015. The incidence of cotton leaf curl virus disease during the experimental year *i.e.* 2015 was very severe under field condition particularly nearby Hisar including the experimental area. During the year 2015 no variety / strain was observed completely immune to this disease. Even in highly resistant / tolerant strains few plants showed susceptible reaction. The F_1 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 F_2 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.

Key words: CLCuD, inheritance, resistant, susceptible, whitefly

Cotton is a major fiber crop of global importance and has high commercial value. The leading cotton producing countries are China, USA, India and Pakistan where climatic conditions suit the natural growth requirements of cotton. Cotton as a cash crop plays an important role in the industrial activity of the nation and has a unique place in the economy of our country. Low productivity of cotton is mainly due to high incidence of insect pests and diseases caused by fungal, bacterial and viral pathogens. Of these viral diseases alone or in combination with other factors are quite destructive. The diseases caused by *Gemini virus* are of considerable concern as the climate of India is favorable for the growth and spread of its vector *i.e.* whitefly (*Bemisia tabaci*). It transmits *Gemini virus* causing cotton leaf curl

virus disease which poses serious threat to cotton production. Typical symptoms of cotton leaf curl virus disease include upward / downward leaf curling, stunted growth and 'enations'.

In India, cotton leaf curl virus disease was first reported in upland cotton in Sriganganagar area of Rajasthan state and consequently it appeared in Haryana and Punjab states and posed a major threat to its cultivation in northern India. Use of chemicals to control this disease is not economic and effective. Therefore, development of a resistant / tolerant variety or hybrid is the most effective, long term, less expensive and safe method to fight against this disease. The successful exploitation of the source of resistance also requires information on the genetic control for development of resistant variety. Research efforts to develop

resistant varieties / hybrids through conventional / biotechnological approaches along with cultural and management practices are in progress for effective control of this disease. In this regard, the farmers can be aware of the preventive measures regarding the control of cotton leaf curl virus through a sound, viable and dynamic integrated management programme which should include use of tolerant varieties, seed treatment, eradication of weeds and alternate hosts (Abbas *et al.*, 2015).

By using biotechnological tools broad spectrum resistance can be introduced against all viruses present in the field (Farooq *et al.*, 2011). A better understanding of the movement of the pathogen might be useful in future efforts to control the disease (Sohrab *et al.*, 2014). Resistance may be improved through selection in later generations under high inoculum of disease (Hussain *et al.*, 2012). Biological control is a novel approach in crop protection. *Bacillus*, *Pseudomonas* and *Burkholderia* isolates are among those strains that not only promote plant growth, but also provide resistance against diseases such as viral diseases in plants in addition to bacterial or fungal diseases (Ramzan *et al.*, 2016). Hybrids having very good economic traits with high seed cotton yield has been developed (Anjum *et al.*, 2015). Diversity among the genotypes could be utilized for the development of CLCuD resistant lines with increased seed cotton yield along with desirable fiber quality (Saeed *et al.*, 2014). Unless a CLCuD resistant variety is not developed, highly tolerant varieties should be cultivated and losses due to this disease should minimize by increasing plant population and intensive inputs in late planting (Iqbal *et al.*, 2014).

MATERIALS AND METHODS

The present investigation was conducted at Cotton Research Area, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar from *kharif* 2013 to 2015. Four parents which included 2 resistant (GCH 3 and H 1353) and 2 susceptible (RST 9 and HS 6) to cotton leaf curl virus disease were chosen to generate the experimental material for the present study. These 4 parents were used to develop 4 crosses *i.e.* GCH 3 x HS 6 (R x S), GCH 3 x RST 9 (R x S), H 1353 x HS 6 (R x S) and H 1353 x RST 9 (R x S). These crosses were designated as cross I, cross II, cross III and cross IV, respectively and finally 4 generations were generated *i.e.* P₁, P₂, F₁, F₂, BC₁ and BC₂.

The experimental material comprised of 4 generations *i.e.* P₁, P₂, F₁, F₂ and back crosses (BC₁ and BC₂) of 4 crosses were grown in a randomized block design with 4 replications during *kharif*, 2015 at Cotton Research Area of CCS Haryana Agricultural University, Hisar. There were 2 rows of non segregating generations (P₁, P₂ and F₁), 10 rows of F₂ and 4 rows of each back cross 1 and back cross 2 generations. The length of each row was 6 m with a spacing 67.5 x 30 cm. In order to build up heavy inoculum pressure one row of highly susceptible line (HS 6) to cotton leaf curl virus disease was planted at the periphery of the experimental area. Normal cultural practices were followed except no insecticidal spray to control whitefly (*Bemisia tabaci* Genn) population in the field. Reaction of cotton leaf curl virus disease was recorded on all the plants in all replications.

Observations on cotton leaf curl virus disease (CLCuD) : Observation on cotton leaf curl virus disease was recorded under field condition in each replication on all the plants of each of the non segregating generations (P_1 , P_2 and F_1), backcross generations and the F_2 generation. Disease was scored on 0-6 grade (Akhtar *et al.*, 2010) depending upon the response to the cotton leaf curl virus disease.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

- 0= Immune
- 1= Highly resistant; disease incidence (0.1– 10%)
- 2= Resistant; disease incidence (10.1– 20%)
- 3= Moderately resistant; disease incidence (20.1 – 30%)
- 4= Moderately susceptible; disease incidence (30.1 – 40%)
- 5= Susceptible; disease incidence (40.1– 50%)
- 6=Highly susceptible; disease incidence (> 50%)

For testing the agreement of observed frequencies with those expected upon a given hypothesis, the chi- square (χ^2) test of goodness of fit was used.

RESULTS AND DISCUSSION

The incidence of cotton leaf curl virus disease during the experimental year *i.e.* 2015 was very severe under field condition particularly nearby Hisar areas including Cotton Research Area of CCS HAU, Hisar. During the year 2015 no variety/ strain was observed completely

immune to this disease. Even in highly resistant strains few plants showed susceptible reaction. The F_1 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 showed resistance to CLCuD which indicated that resistance is a dominant and digenically controlled 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 dominance nature of resistance over susceptibility was further confirmed by backcrosses and F_2 s, there were 2 distinct classes, *i.e.* resistant and susceptible in F_2 and backcross population. The pattern of segregation in F_2 gave a good fit to 9 resistant : 7 susceptible (Table 1) indicated the complementary type of gene action. Complementary type of gene interaction for CLCuD was further confirmed by a good fit of 3 resistant: 1 susceptible ratio of backcross with susceptible parents.

The segregation in F_2 population of cross I *i.e.* GCH 3 x HS 6 was 240 resistant : 166 susceptible which gave a good fit to the expected 9 resistant: 7 susceptible ratio which indicated the complementary type of gene interaction. These results suggested that plants showed resistant reaction in the presence of dominant alleles of both the genes. Disease was expressed in those plants which had any one of the 2 or both the genes in the homozygous recessive state. These results were further confirmed by the good fit to 3 resistant: 1 susceptible ratio (135 resistant: 39 susceptible observed in the backcross population with the susceptible parent HS 6 (Table 1). Likewise in F_2 generation of cross III *i.e.* H 1353 x HS 6, plants segregated leading to 386 resistant: 320 susceptible. The chi square values showed a good fit to the expected ratio of 9 resistant: 7 susceptible thus provided

Table 1. Inheritance of cotton leaf curl virus disease in upland cotton (*Gossypium hirsutum* L.)

Parent /generation	Number of plants		Expected ratio	a ^{χ2} calculated	P value
	Screened	Resistant			
Cross I (R x S): GCH 3 x HS 6					
P ₁	82	80	2	-	-
P ₂	68	0	68	-	-
F ₁	76	75	1	-	-
F ₂	406	240	166	9:7	1.35
BC ₁	28	26	2	-	-
BC ₂	174	135	39	3:1	0.61
Cross II (R x S): GCH 3 x RST 9					
P ₁	93	90	3	-	-
P ₂	86	0	86	-	-
F ₁	35	34	1	-	-
F ₂	591	340	251	9:7	0.39
BC ₁	54	53	1	-	-
BC ₂	102	74	28	3:1	0.32
Cross III (R x S): H 1353 x HS 6					
P ₁	109	103	6	-	-
P ₂	104	0	104	-	-
F ₁	51	50	1	-	-
F ₂	706	386	320	9:7	0.715
BC ₁	238	233	5	-	-
BC ₂	174	133	41	3:1	0.096
Cross IV(R x S): H 1353 x RST 9					
P ₁	79	73	6	-	-
P ₂	71	0	71	-	-
F ₁	80	77	3	-	-
F ₂	624	335	289	9:7	1.66
BC ₁	158	152	6	-	-
BC ₂	164	119	45	3:1	0.52

the evidence that resistance to CLCuD in H 1353 was governed by dominant alleles of both the genes. For disease appearance any one of the 2 or both the genes should be in homozygous recessive condition. Complementary type of gene interaction for CLCuD was further confirmed by a good fit of 3 resistant: 1susceptible *i.e.* (133 resistant: 41 susceptible) ratio of backcross 2 with susceptible parent HS 6 (Table 1).

Previous work on the inheritance of this viral disease is scanty, and what exists in the literature shows that CLCuD is controlled by a major gene, and single gene with dominant effects. Involvement of a major dominant gene in controlling the resistance of leaf curl virus along with the involvement of minor (modifiers), genes was also revealed. Quantitative inheritance with predominance of additive gene

effects affecting resistance to CLCuD. Inheritance of cotton leaf curl virus disease (CLCuD) was also studied in 4 crosses which involved resistant and susceptible parents to this disease. 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 R x S crosses. In F₂ generation of R x S crosses, the duplicate dominant (15 resistant: 1 susceptible) effect for inheritance of cotton leaf curl virus disease was observed in upland cotton (Godara *et al.*, 2016).The breeding for cotton leaf curl disease resistance has been achieved through the assemblage of minor genes by recurrent selection and resistance depends on major genes (dominant genes) which may lose quickly because of the evolution of pathogen for these genes (Azhar *et al.*, 2010). The F₁ of crosses

between highly susceptible S 12, highly resistant LRA 5166 varieties were found as all virus free plants and their F_2 was close to 3:1 ratios which exhibit the presence of a single gene for the inheritance of resistance against CLCuD. Four types of segregation patterns in the F_2 generations were reported. A good fit for 15 (resistant):1 (susceptible), 13 (resistant):3 (susceptible), 9 (resistant):7 (susceptible) ratios indicated digenic control of the trait with duplicate dominant, dominant inhibitory and duplicate recessive epistasis, respectively.

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