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EXPRESSION OF GENES CONTROLLING THE INHERITANCE OF RESISTANCE TO COTTON LEAF CURL VIRUS DISEASE (CLCuD) IN *GOSSYPIUM HIRSUTUM* L.: A QUANTITATIVE ANALYSIS

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Abstract

Forty cotton genotypes were screened for their responses to cotton leaf curl virus symptoms through epidemiology in field and glasshouse, and grafting inoculation during 2001 and 2002. The CLCuD disease ratings, in glasshouse and field conditions, and grafting classified /categorized the variety NIAB-111 as highly resistant and the exotic genotypes NuCOTN-35B as highly susceptible parents. These two lines were hybridized to develop F_1, F_2, BC_{Nu} (back cross with NuCOTN-35B) and BC_{N1} (back cross with NIAB-111) generations to obtain information on the genetic basis of variation for CLCuD resistance through generation mean analysis. Additive- dominance model was found adequate, and the genes responsible for CLCuD resistance were, in general, dominant to their alleles responsible for CLCuD susceptibility. The narrow sense heritability (h^2_{NS}) was moderately high for CLCuD resistance, which along with the estimates of heterosis and genetic advance suggested a potential for the development of breeding material having resistance to CLCuD. However, use of rigorous selection coupled with evaluation of the resistant plants selected from segregating material under higher viral load is suggested to develop CLCuD resistance in *Gossypium hirsutum* L.

Introduction

Cotton leaf curl virus disease (CLCuD), a whitefly (*Bemisia tabaci* Genn) transmitted Gemini virus (Yassin & El-Nour, 1970; Idris, 1990; Mansoor *et al*., 1993; Hameed *et al*., 1994; and Akhtar *et al*., 2002b) is well known for causing major disasters to cotton production in Nigeria, Tanzania and Sudan (Farquharson, 1912; Bailey, 1934). In Pakistan, occurrence of CLCuD was observed on cotton plants for the first time in District Multan during 1967 (Hussain & Ali, 1975), and until 1986 its consistent persistence on cotton crop was noted with out causing any significant damage. The disease appeared in epidemic form in 1991 and in the subsequent years causing significant reduction in production from 12.82 million bales during 1991 to 8.04 million bales during 1994. During the last decade this disease has caused a loss of 7.1 million bales amounting to 1.2 billion US dollars to national economy (Mahmood, 1999). The extents of losses to cotton production by this disease are still alarming in Pakistan after the appearance of new Burewala CLCuD strain, which caused a break down of resistance in the cotton varieties (Mahmood *et al.,* 2003; Mansoor *et al*., 2003). The epidemic of CLCuD in Pakistan is one of the best examples of the dramatic shift in importance of a previously unimportant disease (Markham, 1992 and Zafar *et al.,* 1997).

To combat this serious disease to cotton culture, with its most pronounced detrimental effects in Pakistan and Sudan, efforts made by the earlier researchers in breeding CLCuD resistant plant material/varieties (Ali, 1997; Hussain *et al*., 2001), signify the effectiveness of the breeding and selection of individual plants from segregating populations under high viral load (Lambart, 1929; Siddiq, 1968; Giha & Nour, 1969). The most susceptible stages of cotton plant to CLCuD (El-Nour & Abu Salih, 1966; El-Nour, 1967 and Akhtar *et al*., 2004), dependency of CLCuD for its temporal pattern spread on factors like, temperature, humidity, vector population, prevailing viral load (Giha & Nour, 1969; Khan *et al*., 1998; Khan & Khan, 2000; Akhter *et al*., 2003; and Sharma & Rishi, 2004), role of driven factors in the spread of tropical whitefly-transmitted gemniviruses of different crops (Fargette *et al*., 1993) in diverse environments have been reported. Diminishing of genetic diversity in new germplasm as major cause of vulnerability of cotton crop to CLCuD (Fouilloux & Bannerot, 1988; Iqbal *et al*., 1997), presence of genetic variability for virulence and responses against CLCuD among the species *i.e*., *G. barbadense* and *G. hirsutum* (Ebbels, 1976), *G. hirsutum* and *G. arboreum* (Perven & Sultan, 2005), and within the species (Akhtar *et al.,* 2002a) deserve for the broadening of genetic base and evolution of CLCuD resistant cotton varieties (Ali, 1999).

In Pakistan, the resistance of CP-15/2, LRA-5166 and Cedix for their CLCuD was transferred into the adapted varieties, which led to the development of two virus resistant varieties, CIM-1100 and CIM-448 at Central Cotton Research Institute, Multan (Pakistan). By the utilization of continuous exotic genetic resources in cotton improvement programme, although an array of new varieties, having resistance to CLCuD were developed but no cultivar having near immunity level of resistance could be developed. Limited success to breed plant material having resistance level as highly resistant or immune level has been resulted due to the lack of informations (i) pattern of variation in CLCuD (ii) comprehensive assessment of its genetic control (iii) changing names of viruses (iv) lack of consistency in viral nomenclature and relatively frequent inter-specific recombination of gemini viruses. 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 (Knight, 1948), and single gene with dominant effects (Ali, 1999; Rehman *et al.,* 2002; Haider, 2002). Involvement of a major dominant gene in controlling the resistance of leaf curl virus along with the involvement of minor (modifiers) genes was revealed by findings of Siddiq (1970). On the contrary, Randhawa

(1999) and Iqbal *et al*., (2003) reported that CLCuD was controlled by two dominant genes and duplicate dominant epistasis, respectively. Findings of Rehman *et al*., (2005) revealed that three genes were involved in *G. hirsutum* resistance to CLCuD, two for resistance ($R_{1CLCuDhir}$ and $R_{2CLCuDhir}$) and a suppressor of resistance ($S_{CLCuDhir}$). On the other hand, Ahuja *et al*., (2006) reported the involvement of two genes with duplicate dominant, dominant inhibitory and duplicate recessive epistasis and three genes with triplicate dominant epistasis in controlling CLCuD resistance. However, the findings of Khan *et al*., (2007) suggested quantitative inheritance with predominance of additive gene effects affecting resistance to CLCuD.

Existence of different strains of cotton leaf curl virus (Zhou *et al*., 1998) in Pakistan suggests the need for thorough studies of genetic mechanism controlling CLCuD, before developing a breeding programme aimed at developing resistant material. Knowledge of the genetic basis and heritability of resistance to CLCuD is essential for the development of resistant cultivars. Thus, the present study was designed to determine the type of gene action controlling the inheritance of resistance to this CLCuD through generation mean analysis using disease rating scales, 0-6 grade (Table 1), developed by Akhtar *et al*., (2001).

Table1. Rating scale for disease reaction to cotton leaf curl virus disease (CLCuD).

Rating	Symptoms	Disease reaction
0.	Complete absence of symptoms	Immune
1.	Thickening of few small scattered veins or only presence of leaf enations on ten or less than ten leaves of a plant after careful observations	Highly resistant
2.	Thickening of small group of veins	Resistant
3.	Thickening of all veins but no curling of leaves	Moderately resistant
4.	Severe vein thickening and mild leaf curling at the top of plant	Moderately susceptible
5.	Severe vein thickening and leaf curling on half of plant	Susceptible
6.	Severe vein thickening, leaf curling and stunting of the plant with no or less fruit bearing	Highly susceptible

Materials and Methods

Assessment of variability for CLCuD resistance: natural inoculum transmission

Plant material and growing conditions: Seeds of 40 cotton genotypes including commercial varieties and nine exotic genotypes *i.e.,* NuCOTN-35B, Reba P-279, Reba B-50, MK-73, (HAR ×BJA)-1186, BJA-592, HAR-444-2, SRI F4-71, and Reba P-288 were collected from various Cotton Research Institutes/Centers in Pakistan. The 40 diverse genotypes were evaluated for their responses to CLCuD under natural field conditions, controlled glasshouse conditions and through artificial graft inoculation techniques. The work presented here was conducted as a part of PhD thesis research in the Department of Plant Breeding and Genetics University of Agriculture, Faisalabad (Pakistan)

Field evaluation: The seeds of 40 cotton genotypes were planted in a field, where cotton was planted in rotation with wheat for at least last five years, in three replications following randomized complete block design in 2001. The field had high natural CLCuD inoculum load. The seeds of each cotton genotype were sown in two rows of 5.4 meter each with intra row space of 75 cm. Thirteen seeds were planted in each row with plant spacing of 45 cm. Observations for CLCuD symptom development and severity on cotton plants were recorded at 35, 70, 105 and 140 days after planting (DAP) using the modified disease scale 0 to 6 (Table 1) previously used by Akhtar *et al*., (2001). The mean disease rating values measured for 40 cotton varieties under field conditions are given in Table 2.

Glasshouse evaluation: The seeds of the 40 cotton genotypes were planted in pots of 30 cm diameter kept in an insect-proof glasshouse. In the glasshouse, temperature was maintained at 30/25 ± 2°C day/night, and plants were exposed to natural sunlight supplemented with artificial light to maintain photoperiod of 16 h. The pots were filled with soil taken from the cotton experimental field area. The soil was sandy (57.8%) - clay (22.7%) - loam (19.5%), with a pH of 7.9, EC 1.2 dS/m, SAR 2.88, organic matter 2.3%, water holding capacity 35.5 %, bulk density 1.5 g/cm and porosity 44.5 %. Six seeds were planted in each pot and at 3-4 leaf stage, seedlings were thinned to two seedlings per pot. In total, there were 10 pots for each cotton genotype. Each pot was fertilized with nitrogenous fertilizer (urea @ 9 g/pot) once a month and clean tap water was applied to the growing seedlings, as required during the plant development. During the experimentation, production of viral inoculum under glasshouse was maintained from naturally infected cotton plants of S-12, an old variety reported to be highly susceptible to leaf curl virus disease (Ali *et al*., 1992). For rapid transmission of CLCuD to the healthy plants, diseased plants of S-12 (spreader line) were placed between plants of the 40 cotton genotypes, and maximum/ minimum temperature and humidity levels required at different plant growth stages matching the field conditions were maintained. Genotypic responses to the disease were recorded by: (1) days taken for appearance of first disease symptom on the plants, and (2) disease reaction by making observations several times at 35, 70, 105 and 140 days after their sowing on the basis of severity of symptoms using 0 to 6 disease rating scale (Table 1).

Table 2. Responses of 40 diverse cotton genotypes to CLCuD grown in the glasshouse and natural field conditions.

Name of cotton accessions	Source	Glasshouse conditions		Natural field conditions		Mean values with ranked order	Disease reaction
		Mean latent period* (days)	Average disease severity after 35, 70, and 105 days of sowing	Mean latent period* (days)	Average disease severity after 35, 70, and 105 days of sowing		
S-14	CRS, Sahiwal, Pakistan	15	6.0 + E	14	6.0 + E	6.0 + E a	HS
S-12	CCRI, Multan, Pakistan	15	5.8 + E	14	5.8 + E	5.8 + E a	HS
Reba P-279	CIRAD de Montpellier, France	14	5.7 + E	16	5.7 + E	5.7 + E ab	HS
NIAB-86	NIAB, Faisalabad, Pakistan	15	5.7 + E	14	5.7 + E	5.7 + E ab	HS
Reba B-50	CIRAD de Montpellier, France	14	5.7 + E	15	5.7 + E	5.7 + E ab	HS
NuCOTN-35B	Georgia, USA	14	5.7 + E	15	5.7 + E	5.7 + E ab	HS
MK-73	CIRAD de Montpellier, France	14	5.6 + E	16	5.6 + E	5.6 + E ab	HS
(HAR×BJA)-1186	CIRAD de Montpellier, France	14	5.6 + E	16	5.6 + E	5.6 + E ab	HS
BJA -592	CIRAD de Montpellier, France	15	5.3 + E	17	5.3 + E	5.3 + E b	S
HAR-444-2	CIRAD de Montpellier, France	15	5.3 + E	17	5.3 + E	5.3 + E b	S
CIM-240	CCRI, Multan, Pakistan	14	5.3 + E	18	5.3 + E	5.3 + E b	S
FH-87	CCRI, Multan, Pakistan	14	5.3 + E	17	5.3 + E	5.3 + E b	S
GOHAR-87	CRS, Multan, Pakistan	16	5.3 + E	18	5.3 + E	5.3 + E b	S
SRI-F4-71	CIRAD de Montpellier, France	17	5.3 + E	19	5.3 + E	5.3 + E b	S
NIAB-Karishma	NIAB, Faisalabad, Pakistan	15	5.3 + E	17	5.3 + E	5.3 + E b	S
CIM-109	CCRI, Multan, Pakistan	17	4.3 + E	19	4.3 + E	4.3 + E c	MS
MNH-147	CRS, Multan, Pakistan	18	4.3 + E	16	4.3 + E	4.3 + E c	MS
FH-682	AARI, Faisalabad, Pakistan	19	4.3 + E	17	4.3 + E	4.3 + E c	MS
SLS-1	CRS, Sahiwal, Pakistan	19	4.3 + E	20	4.3 + E	4.3 + E c	MS
NIAB-78	NIAB, Faisalabad, Pakistan	16	4.0 + E	18	4.0 + E	4.0 + E c	MS
MNH-329	CRS, Multan, Pakistan	18	4.0 + E	20	4.0 + E	4.0 + E c	MS
BH-36	CRS, Bahawalpur, Pakistan	17	4.0 + E	18	4.0 + E	4.0 + E c	MS
NIAB-98	NIAB, Faisalabad, Pakistan	19	3.0 + E	18	3.0 + E	3.0 + E d	MR
NIAB-94	NIAB, Faisalabad, Pakistan	19	3.0 + E	22	3.0 + E	3.0 + E d	MR
NIAB-801	NIAB, Faisalabad, Pakistan	19	3.0 + E	25	3.0 + E	3.0 + E d	MR
CIM-448	CCRI, Multan, Pakistan	17	3.0 + E	19	3.0 + E	3.0 + E d	MR
Reba P-288	CIRAD de Montpellier, France	19	3.0 + E	20	3.0 + E	3.0 + E d	MR
CIM-482	CCRI, Multan, Pakistan	18	2.6 + E	19	2.6 + E	2.6 + E de	MR
CIM-473	CCRI, Multan, Pakistan	18	2.3	23	2.3	2.3 ef	R
NIAB -388	NIAB, Faisalabad, Pakistan	24	2.0	28	2.0	2.0 fg	R
NIAB -588	NIAB, Faisalabad, Pakistan	25	2.0	27	2.0	2.0 fg	R
CIM-443	CCRI, Multan, Pakistan	18	2.0 + E	19	2.0 + E	2.0 + E fg	R
CIM-446	CCRI, Multan, Pakistan	18	2.0 + E	20	2.0 + E	2.0 + E fg	R
FH-634	AARI, Faisalabad, Pakistan	17	2.0 + E	20	2.0 + E	2.0 + E fg	R
FH-900	AARI, Faisalabad, Pakistan	22	1.8	25	1.8	1.8 g	R
FH-901	AARI, Faisalabad, Pakistan	20	1.8	24	1.8	1.8 g	R
FH-945	AARI, Faisalabad, Pakistan	22	1.8	25	1.8	1.8 g	R
NIAB-358	NIAB, Faisalabad, Pakistan	28	1	29	1	1.0 h	HR
CIM-1100	CCRI, Multan, Pakistan	25	1	28	1	1.0 h	HR
NIAB-111	NIAB, Faisalabad, Pakistan	35	0.06	†	0	0.03 i	HR

*= Time taken for first disease appearance; E= Foliar outgrowths (enation); †= no CLCuD symptom appeared till maturity
HS= Highly susceptible; S= Susceptible; MS= Moderately susceptible; MR= Moderately resistant; R= Resistant; HR= Highly resistant

Artificial inoculum transmission through grafting: The seeds of nine CLCuD resistant cotton varieties viz., NIAB-111, Mutant-358, Mutant-588, FH-900, FH-901, CIM-443, CIM-482, and CIM-473 and CIM-1100 and two CLCuD susceptible genotypes viz., S-12 and NuCOTN-35B, selected on the basis of their responses to CLCuD under field and glasshouse conditions in the preceding experiments, were sown in soil filled earthen pots of 30 × 25 cm diameter and depth respectively during January, 2002 under controlled conditions in glasshouse. Temperature, light and photoperiod was maintained artificially as explained in above paragraps. For CLCuD inoculum transmission, 10 plants from each genotype were grafted using the "bottle shoot grafting method'' to study their responses to high inoculum loads of CLCuD. Grafting involved making a 1-2 cm long × 0.1-0.2 cm deep cut on the stem near the second last internode of 6-week-old plants. A similar cut on the CLCuD infected branch with a growing tip of about 20-25 cm long detached from the diseased plant of S-12 was made. The corresponding cut surfaces were bound together and tied with parafilm to avoid drying and to stop air entry. Care was taken to bring the corresponding cambium surfaces into contact. This stem was then placed in 16 cm long test tube with 2 cm diameter filled with distilled water. Distilled water was changed daily at 12-13 noon for 5 days. After 5 days, these tubes were removed and plants were observed daily to see the success for disease transmission.

Development of plant material for the quantitative analysis of CLCuD resistance: Two cotton genotypes NIAB-111 and NuCOTN-35B assessed as most resistant and susceptible to CLCuD respectively, in the preceding experiments were hybridized to develop F_1, F_2, BC_{Nu} and BC_{N1} generations during normal crop season, 2002 and under controlled conditions during December-January, 2002-03. The seeds of each of the six generations i.e., NuCOTN-35B (P_1), NIAB-111 (P_2), F_1, F_2, BC_{Nu} and BC_{N1} generations were divided into two equal halves, one to be evaluated in the field and other in the glasshouse for CLCuD resistance.

Evaluation of genetic material under field conditions: The seeds of the six families i.e., NuCOTN-35B (P_1), NIAB-111 (P_2), F_1, F_2, BC_{Nu} and BC_{N1} were sown following triplicated randomized complete block design in the field where inoculum of CLCuD prevailed naturally during normal crop season, 2003. The seeds were dibbled 45 cm apart in 5.4 meter long rows spaced 75 cm apart. There was one row for each of P_1, P_2 and F_1 generation, 10 rows of F_2 generation, and 5 rows of each BC_{Nu} and BC_{N1} generations. In total 30 plants for each of P_1, P_2, and F_1, 270 plants of F_2, and 150 plants of each BC_{Nu} and BC_{N1} were raised in three repeats. The responses of individual plants of six generations to CLCuD were observed at 4, 7, 10, 12 and 15 weeks of plant age and rated according to scale given in Table 1. The soil in which this experiment was carried out, was sandy-clay loam (58: 23: 19 sand, silt, clay respectively) in its nature. Its pH 7.9, EC 1.2 dS/m, SAR 2.88, organic contents 2.3%, total N 0.06%, water holding capacity 35.5%, bulk density 1.5 g/cm and porosity 44.5%. The standard agronomic practices followed were preparation of land, thinning of seedlings and dry hoeings (2-3). First irrigation was applied after 45 days of planting followed by 4 consequent irrigations with interval of 15 days of considering crop requirements. Fertilizers i.e. NPK was applied @ standard recommended doses *i.e*., (DAP @ 2.5 bags/ hectare; Urea; 3-6 bags/ hectare and Potash (K_2SO_4) @ 70 Kg/ hectare). Appropriate plant protection measures were also used when required.

Evaluation of genetic material under glasshouse conditions: The seeds of the six generations were also planted in soil filled earthen pots under the insect-proof glasshouse during normal crop season, 2003. The study was carried out using procedure of Tripathi & Varma (2003). After emergence at 3-4 leaf stage, seedlings were thinned to five seedlings of each P_1, P_2 and F_1, six seedlings of F_2, and five seedlings of each BC_{Nu} and BC_{N1} in each pot. In total there were 15 plants of each non-segregating population i.e. P_1, P_2, F_1, 270 plants of F_2 and 150 plants of each BC_{Nu} and BC_{N1} generations, which were grown following CRD design with three repeats. The CLCuD inoculum was produced and maintained as explained in preceding paragraphs. The data was recorded using severity symptoms explained in Table 1.

Statistical analyses: Differences in CLCuD resistance among accessions were tested with a generalized linear model analysis of variance (SPSS 8.0 for windows: Advance Statistics, 1994). Statistically significant means were separated with a Tukey's Honestly Significant Difference (HSD) test. A weighted least square analysis of generation means was performed following Mather & Jinks (1982). Model fitting was commenced with the simplest model ('m' only) to increasing complexity (md, mdh, etc.). The best fitted model was one, which had significant parameters along with non-significant chi square value. Narrow-sense heritability (h^2_{NS}) estimates were computed using function of Warner (1952). Heterosis and inbreeding depression were estimated following Mather & Jinks (1982), whilst expected genetic advance was computed using formula given by Falconer & Mackay (1996). The potence ratio, a measure of average degree of dominance (hp), was calculated using the formula given by Griffing (1950).

Results

Variability for CLCuD resistance: The responses of 40 cotton varieties/lines to CLCuD were different but disease ratings under field and glasshouse conditions were similar. Average response of 22 cotton genotypes to CLCuD across two growing conditions was rated from 4.03 to 6.00 and in contrast that of 18 genotypes was rated from 0.03 to 3.00 (Table 2). There was 100% success in grafting and transmission of CLCuD infection in 11 cotton varieties through grafting technique (Table 3). Average disease severity after 70 days of grafting was rated at 1 for four varieties, 2 for five varieties and 6 for two varieties. First CLCuD symptom appeared as early as 14 days after grafting in S-12 and NuCOTN-35B, and that as late as 35 days after grafting in NIAB-111 (Table 3).

Table 3. Responses of cotton genotypes to CLCuD infection through artificial grafting technique after 70 days of grafting.

Cotton varieties	Grafting success (%)	Infectivity	Mean latent period* (days)	Average disease severity	Disease reaction
NIAB-111	100	100	35	1	HR
NIAB-358	100	100	30	1	HR
NIAB-588	100	100	29	1	HR
CIM-1100	100	100	27	1	HR
FH-901	100	100	25	2	R
FH-900	100	100	24	2	R
CIM-473	100	100	17	2	R
CIM-482	100	100	18	2+E	R
CIM-443	100	100	19	2+E	R
S-12	100	100	14	6+E	HS
NuCOTN-35B	100	100	14	6+E	HS

*= Time taken for first disease appearance; E=Foliar outgrowths (enation); HR=Highly resistant; R=Resistant; HS=Highly susceptible

Genetic basis of CLCuD resistance: The two growing conditions were statistically non-significant, and P_1 (NuCOTN-35B), F_2 and BC_{Nu} were significantly ($p \leq 0.01$) different with each other and from P_2, F_1, and BC_{N1} (Fig. 1). The Generations P_2, F_1, and BC_{N1} were non-significantly ($p > 0.05$) different with each other (Fig. 1).

Estimates of gene effects in the individual cross clearly illustrate the variation (Table 4). Additive, and dominance gene effects were statistically significant. F_1 hybrid NuCOTN-35B × NIAB-111 displayed non-significant better parent heterosis of 0.10 (Table 5). F_2 displayed non-significant negative inbreeding depression (-0.88). The potence ratio was negative and less than 1. The magnitude of genotypic and phenotypic variances is close i.e., 3.38 and 3.83 respectively. Estimates of narrow-sense heritability and genetic advance are 0.75 and 2.83, respectively.

Table 4. Estimates of different types of gene effects for average severity of CLCuD over 35, 70 and 105 days after sowing in a cross between resistant and susceptible cotton varieties.

Gene effects	Estimates
m	3.06 ± 0.11
[d]	2.04 ± 0.12
[h]	-2.37 ± 0.21
[i]	NS
[j]	NS
[l]	NS
χ^2	3.57^{NS}
DF	2
Probability	0.17

m, mean; [d], additive effects; [h], dominance effects; [i], additive × additive epistatic effects; [j], additive × dominance epistatic effects; and [l], dominance × dominance epistatic effects
DF and NS indicate degrees of freedom and non-significance at $p > 0.05$ respectively

Table 5. Estimates of heterosis, potence ratio (hp), inbreeding depression, genotypic variance $(\hat{\sigma}^2_g)$, phenotypic variance $(\hat{\sigma}^2_p)$, environmental variance $(\hat{\sigma}^2_e)$, narrow-sense heritability (h^2_{NS}) and genetic advance (GA) for average severity of CLCuD over 35, 70, 105 days after sowing in a cross between resistant, and susceptible cotton varieties.

Parameters	Estimates
Heterosis	0.10 ± 1.61
Inbreeding depression	-0.88 ± 1.55
hp	-0.94
$\left(\hat{\sigma}^2_g\right)$	3.38
$\left(\hat{\sigma}^2_p\right)$	3.83
$\left(\hat{\sigma}^2_e\right)$	0.45
(h^2_{NS})	0.75
GA (NS)	2.83

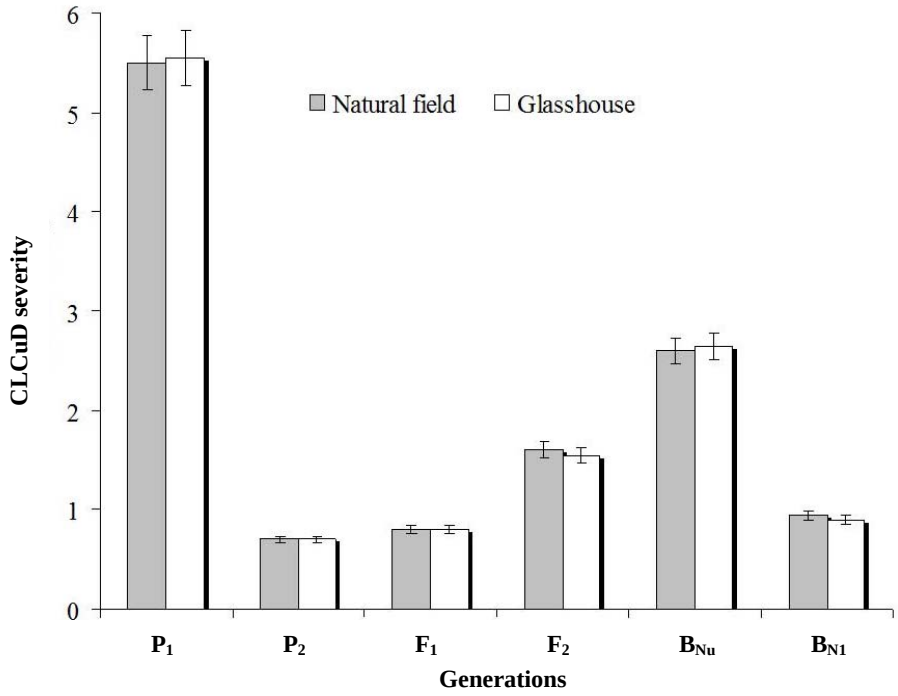

Fig. 1. Average severity of CLCuD over 35, 70 and 105 days after sowing in six cotton generations grown in natural field and glasshouse conditions.

Discussion

Availability of the potential parents is necessary before breeding CLCuD resistant plant material through selection. In the present study, three cotton varieties/lines viz., NIAB-111, CIM-1100 and NIAB-358 appeared to be highly resistant to CLCuD. NIAB-111 exhibited least disease rating of 0.06 and zero under glasshouse and natural field conditions respectively. First symptom of CLCuD appeared earlier on plants of susceptible varieties/lines than resistant one (Table 2). In total 18 varieties/lines were seemed to be CLCuD resistant in this study. Most of them are modern cultivars grown in the cotton belt. Among them are CIM-1100, a CLCuD resistant cotton cultivar evolved through breeding using exotic resistant genetic resources, and an array of cotton varieties namely CIM-435, CIM-443, MNH-552, CIM-446, CIM-448, CIM-482, and FH-634, FH-901 and FH-1000 developed at different research centers after CIM-1100. Retesting of few of them substantiated their resistance to CLCuD when infected through grafting (Table 3). Although these varieties showed resistance against CLCuD as symptoms of the disease appeared at low severity rate in the present study, yet these exhibited severe disease symptoms under farmer's fields in Burewala during recent past. The susceptibility of resistant varieties under farmer's fields might be due to infection by different strains of virus or Burewala climate is more suitable for viral infection and proliferation. The existence of different strains of CLCuD in Pakistan had already been reported by Zhou *et al*., (1998). However, Briddon *et al*., (2000) and Rahman *et al*., (2001) reported poor adaptability/response of cotton varieties to the prevailing incidence of CLCuD in the cotton belt. Mansoor *et al*., (2003) suspected that CLCuD resistance in modern cotton cultivars has been broken down.

Availability of differing accession responses to CLCuD would be good, if it is genetically controlled. Additive-dominance model was found adequate for the present genetic material. More or less equal relative values of [d] and [h], and negative [h] indicates presence of dominance and that genes responsible for CLCuD resistance are in general dominance to their alleles responsible for CLCuD susceptibility. Additive genetic variation had been suggested as being of greater importance in traits which are less complex in their inheritance (Gamble, 1962a, 1962b). Positive better parent heterosis and negative inbreeding depression suggest careful and rigorous selection in the segregating generations. Thus based upon the gene action, high narrow-sense heritability and genetic advance for CLCuD resistance, it seems that resistance may be improved through selection in later generations under high inoculum of disease, as had been done in Sudan (Giha & Nour, 1969), where research workers adopted a strategy of making continuous selection of the resistant plants from the available variability. Therefore, further evaluation of the selected plants from segregating material is suggested in the follow-up programme aiming to develop CLCuD resistance in *Gossypium hirsutum* L.

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