Inheritance of Resistance to *Cotton Leaf Curl Virus* in Cotton (*Gossypium hirsutum* L.)

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Abstract

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Resistance to *Cotton leaf curl virus* (CLCuV) in three cultivars of cotton was investigated in crosses with a susceptible cultivar using generation mean analysis. No single gene of major effect controlled resistance to *Cotton leaf curl virus* in the three crosses. The mean number of effective factors controlling resistance in cross LRA-5166 × S-12 was estimated to be at least five. Estimates of broad and narrow sense heritability indicate that effects by the environment were larger than those of genetic components. Epistasis was significant in two crosses. Additive gene effects contributed more to resistance than to susceptibility in contrast with dominance gene effect. Reciprocal differences were detected in the cross with LRA-5166. Estimates of genetic gain ranged form low to moderate. Thus, a breeding method that makes use of additive variance should be used because much of the variances for resistance are additive, whereas dominance effects, at least in these crosses, tended to contribute to susceptibility.

Keywords: *Cotton leaf curl virus*; *Gossypium hirsutum*; heritability of resistance; generation mean analysis

Cotton leaf curl virus (CLCuV) on cotton (*Gossypium hirsutum* L.) is characterised by thickening of veins and curling of leaves, under severe attack it produces foliar outgrowth, i.e. enations. It is transmitted by whitefly (*Bemisia tabaci* Gem.) and belongs to the genus *Begomovirus* (family Geminiviridae), Gemini virus subgroup III (Hameed *et al.* 1994). In Pakistan, the disease was first observed near the cotton belt of Multan (Punjab) on a few cotton plants in 1967. Since the disease was of minor importance, it did not attract serious attention although it had been noticed subsequently. Yet in 1992–1993 it appeared in epidemic form, which decreased cotton yield to 9.05 million bales and further decreased it to 8.04 million bales during 1993–1994. The CLCuV disease caused a reduction of 7.1 million bales (during the last decade); which caused a loss of 1.2 billion dollars to the national economy (Mahmood 1999). Resistant varieties are the only permanent solution to the problem. During the last decade, considerable efforts have been made by various research organisations to develop cultivars resistant to CLCuV. Field observations proved that some commercial cultivars showed different levels of resistance to CLCuV (Akhtar *et a*l. 2001, 2002). However, there has been no comprehensive assessment of the inheritance of this resistance in cotton. Knowledge of the genetic basis and heritability of resistance to CLCuV is essential for the development of resistant

cultivars. Thus, the present study was designed to determine the type of gene action controlling resistance, the genetic and environmental components of variance, estimates of heritability and gain from selection, estimates of the minimum number of the effective factors controlling resistance and to investigate the effect of cytoplasmic inheritance on resistance.

MATERIAL AND METHODS

The parents were selected based on their diversity and previously reported level of resistance (Akhtar *et al*. 2001, 2002; Mahmood *et al.* 2003). The resistant parents were FH-900, CIM-448, LRA-5166; the susceptible parent was S-12.

Crosses. Crosses were made between resistant and susceptible parents as follows, FH-900 × S-12, CIM-448 × S-12 and LRA-5166 × S-12. Generation mean analysis was performed using each resistant (P_1) and susceptible parent (P_2), F_1 and F_2 generations including reciprocals (F_1' and F_2') and backcrosses of the F_1 to each parent (BCP_1 and BCP_2).

Source of viral inoculum and maintenance of cultivars. The viral inoculum used in this study consisted of CLCuV infected cotton plants that were maintained in the greenhouse at the Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad, Pakistan.

Field test. The field test was conducted at the Department of Plant Breeding & Genetics, University of Agriculture, Faisalabad. It was planted on 6 June 2003 and repeated on 9 June 2004. The test was carried out during the summer season with an average temperature of 40°C.

Pathogen transmission. Six-week-old plants were selected for graft transmission of CLCuV from infected plants, using the leaf-petiole graft technique as described by Akhtar *et al*. (2000). A cut 0.5 cm long and 0.1 cm deep was made on the stem of the plant to be tested. A leaf was detached from a CLCuV infested plant, and a similar cut was made on the petiole of that isolated leaf. The corresponding cuts were brought together, taking care to bring the corresponding cambium surfaces into contact. Para-film was then tied around stem and petiole to keep them from drying out and to stop the entry of air. The bottom end of the leaf petiole was placed in a test tube with distilled water that was changed daily around 12–13 h for 5 days. The tubes were then removed and the plants observed daily to determine disease transmission and symptoms. In both years, individual plants were rated 1 week after inoculation by using the modified scale (Table 1) and the rating continued up to 60 days. Grafting was done on 20 plants of parents and F_1 generations of each cross, while in the F_2 generations and back crosses, grafting was done on 80 and 40 plants, respectively, in each cross. Infectivity or success of grafting was 100% in all cases.

Experimental design and data analysis. The experimental design for all experiments was a randomised complete block design with two replicates. Plants were spaced 30 cm apart in rows separated by 75 cm.

Table 1. Modified disease scale for the rating of CLCuV disease

Rating	Symptoms	Disease incidence (%)	Disease reaction
0	complete absence of symptoms	0	immune
1	very minute thickening of veins	0.1–5.0	very highly resistant
2	thickening of some scattered veins	5.1–10.0	highly resistant
3	thickening of small group of veins	10.1–20.0	resistant
4	thickening of all veins	20.1–30.0	moderately resistant
5	severe vein thickening and leaf curling developed at the top of the plant	30.1–50.0	moderately susceptible
6	severe vein thickening and leaf curling developed on the half of the plant canopy	50.1–75.0	susceptible
7	severe vein thickening, leaf curling and full stunting of the plant	75.1–100.0	highly susceptible

Analyses of variance indicated that year and replication effects were non-significant, and transforming the data by square root or arcsin had no effect on the additivity of the scale or distribution of the data. Additive effect (σ^2_A), dominance (σ^2_D) and narrow (h^2) sense heritability were estimated by the method of Warner (1952). Environmental (σ^2_E) variances were estimated as done by Wright (1968). Broad sense heritability was estimated as $H = \sigma^2F_2 - \sigma^2D/\sigma^2F_2$. Gene effect based on a six parameter model was estimated using the non-weighted method as described by Gamble (1962). The number of effective factors controlling resistance was estimated by five methods. Method 1 followed that by Wright (1968), method 2 had been proposed by Mather and Jinks (1982) and methods 3, 4 and 5 had been prepared by Lande (1981). All effective formulas assume that segregating genes for resistance are all allocated in one parent, resistant genes are not linked, all resistant genes have equal effect on resistance, epistasis dominance and genotype × environment effects are absent (Wright 1968).

RESULTS

Means and their SE (standard error) for parental, F_1, F_2 reciprocal and backcross generations are listed in Table 2. Of the parents, LRA-5166 was most resistant, followed by CIM-448 and FH-900, while S-12 was highly susceptible. Heterosis was negative and toward the resistant parent in two crosses, i.e. FH-900 × S-12 and LRA-5166 × S-12, while in cross CIM-448 × S-12 it was positive and toward the susceptible parent. No significant differences were found between F1 ($P_1 \times P_2$) and

Table 2. Leaf curl rating means ± SE for resistance to CLCuV disease of parents and offspring populations from three crosses

Population[1]	CIM-448 (R) × S-12 (S)	FH-900 (R) × S-12 (S)	LRA-5166 (R) × S-12 (S)
P_1	3.13 ± 0.24^d	4.22 ± 0.12^d	2.11 ± 0.10^h
P_2	6.18 ± 0.20^a	6.18 ± 0.20^a	6.18 ± 0.20^a
F_1	5.19 ± 0.15^c	5.31 ± 0.13^b	4.14 ± 0.21^d
F_2	5.67 ± 0.14^b	5.36 ± 0.22^b	3.73 ± 0.18^f
BC_1	5.72 ± 0.21^b	5.11 ± 0.22^c	2.44 ± 0.12^g
BC_2	6.01 ± 0.19^a	6.11 ± 0.29^a	6.77 ± 0.37^b
F_1'	5.21 ± 0.19^c	5.36 ± 0.17^b	4.31 ± 0.22^c
F_2'	5.68 ± 0.15^b	5.39 ± 0.18^b	4.01 ± 0.19^e

[1]Population notation (Female listed first in each cross); P_1 = resistant parent; P_2 = susceptible parent; F_1 ($P_1 \times P_2$); $F_1' = (P_2 \times P_1)$; BC_1 ($F_1 \times P_1$); BC_2 ($F_1 \times P_2$); F_2 ($F_1 \times F_1$); $F_2 = (F_1' \times F_1')$
Means followed by the same letter are not significantly different at $P < 0.05$

Table 3. Estimates of gene effects ± SE for resistance to CLCuV disease in three crosses

Population	CIM-448 (R) × S-12 (S)	FH-900 × S-12 (S)	LRA-5166 (R) × S-12 (S)
m	5.67 ± 0.14**	5.36 ± 0.22**	3.73 ± 0.18**
a	0.33 ± 0.20	–3.41 ± 0.27**	–1.71 ± 0.21**
d	0.68 ± 0.92	–4.31 ± 1.10**	–1.51 ± 0.20**
aa	1.31 ± 0.90	–4.82 ± 0.83**	0.91 ± 0.56
ad	1.21 ± 0.21**	–1.67 ± 0.40**	0.01 ± 0.37
dd	–2.62 ± 1.21	7.67 ± 0.78**	–0.66 ± 0.38

** Estimates significantly different from zero at $P = 0.01$

Table 4. Estimates of additive (σ^2_A), dominance (σ^2_D) and environmental (σ^2_E) variances, broad (H) and narrow (h^2) sense heritability and genetic gain through selection (G_s) for resistance to CLCuV

Cross	σ^2_A	σ^2_D	σ^2_E	H	h^2	G_s
CIM-448 × S-12	–2.91	3.11	0.71	0.32	0.09	0.03
FH-900 × S-12	1.66	–0.93	1.92	0.27	0.57	1.81
LRA-5166 × S-12	0.71	–0.28	1.82	0.17	0.29	0.83

Table 5. Estimates of the minimum number of genes or effective factors (EF) controlling resistance to CLCuV in three crosses

Cross	EF_1	EF_2	EF_3	EF_4	EF_5	Mean
CIM-448 × S-12	1.11	–0.21	0.83	–0.10	0.12	0.35
FH-900 x S-12	1.92	1.21	1.92	0.83	–1.72	0.83
LRA-5166 × S-12	4.21	4.33	3.9	3.4	10.12	5.19

$F_1'(P_2 \times P_1)$ or between F_2 $(F_1 \times F_1)$ and $F_2'(F_1' \times F_1')$ of crosses CIM-448 × S-12 and FH-900 × S-12. In cross LRA-5166 × S-12, however, there were significant differences between the reciprocals in both the F_1 and F_2 generation; the F_1 and F_2 were more resistant than their reciprocals. Similarly, the F_1 generation was more resistant than the F_2 in cross CIM-448 × S-12; whereas the difference was not significant in cross FH-900 × S-12 and the F_2 generation was found more resistant than the F_1, capable of giving maximum transgressive generations. The mid point ranged from 3.73 to 5.67 (Table 3), it was lowest for cross LRA-5166 × S-12. The gene effect showed the presence of non-allelic interactions (epistasis) in crosses CIM-448 × S-12 and FH-900 × S-12 (Table 3). The additive × dominance type of digenic interaction was important for cross CIM-448 × S-12 while all three types of digenic interactions were significant in cross FH-900 × S-12. The magnitude of a dominant genetic effect was highest in cross FH-900 × S-12, while the magnitude of an additive effect was highest in cross LRA-5166 × S-12 (Table 3). The environmental component of variance was larger than the additive or dominance variances in crosses LRA-5166 × S-12 and FH-900 × S-12, while the dominance variance was greater in cross CIM-448 × S-12 (Table 4). Additive variance was greater in magnitude than dominance in crosses FH-900 × S-12 and LRA-5166 × S-12 (Table 4). Heritability estimates varied between crosses (Table 4). Broad sense heritability ranged from 0.17 to 0.32. Genetic gain per cycle for selection of 10% level (Gs) ranged up to 1.81. Estimates of the minimum number of genes controlling CLCuV resistance are presented in Table 5. Estimates over all crosses ranged from –0.10 to 10.12; mean estimates for individual crosses ranged from 0.35 to 5.19.

DISCUSSION

In none of the crosses was there complete dominance or a distinct bimodal distribution, suggesting that resistance is not controlled by a single gene of major effect. Dominance and most types of epistasis will bias an estimate of effective factors (Wright 1968). It is likely that estimates of the number of effective factors were highly biased by failure to meet the analysis assumptions of no epistasis and no dominance, because dominance effects were present in the crosses and epistasis was significant except in cross LRA-5166 × S-12. This cross, in addition to lacking epistasis, was lower in dominance. Therefore, the estimates of the minimum effective factors of that cross are likely to be more accurate than the estimates of other crosses. The generally low estimates of broad and narrow sense heritability indicated that the environment in which the parents are tested has a larger effect on leaf rating than their genotypes. This finding is in agreement with the low realised heritability for mass selection reported by Wyszogrodzka *et al.* (1986). Thus, breeding efforts

to increase resistance will require good control over environmental variations. In general, additive variance was larger than dominance variance. Significant estimates of additive gene effects were usually negative, indicating that additive effects contribute more to resistance than to susceptibility. A reciprocal difference consistent with cytoplasmic inheritance was detected in cross LRA-5166 × S-12. Used as maternal parent, LRA-5166 produced more resistant offsprings than if used as paternal parent. The genetic gain for resistance to CLCuV was low to moderate when using the resistance found in CIM-448, FH-900 and LRA-5166. In such cases, a breeding method such as progeny to row selection should be used because it makes use of additive variance. Additionally, methods that provide better control over the environment should be adopted because environmental variation is larger than additive variance. Cytoplasmic effects in cotton should be investigated further, especially in the context of CLCuV resistance.

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