# Studies on the Cotton Leaf Curl Virus (CLCuV) disease in north India

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## ABSTRACT

*Cotton Leaf Curl Virus (CLCuV) disease caused by a white fly transmitted Gemini virus has assumed serious proportions in the irrigated cotton belt of north India. Identification of sources of resistance and development of a detection system are important steps in the effective management of this important disease. In the present studies, standardization of a screening methodology, identification of resistant lines and detection of CLCuV disease using antiserum has been attempted. Screening of germplasm lines against CLCuV disease was carried out under field conditions from 1997 to 2001 and between 1040 and 1531 lines were screened each year during the period. Based on five years of screening, sixty-five resistant lines were identified. In order to develop a screening nursery, two rows of leaf curl susceptible variety F-846 and one row of white fly susceptible variety HS-6 were planted on three sides of a 0.4 ha field. This hedge with leaf curl infected plants was pruned in the off-season to maintain the inoculum of the disease. Up to 90% disease incidence was noted in this screening nursery. The following antisera (i) ACMV coat protein fusion protein antisera,(ii) Antiserum raised against purified ACMV virions (iii) Antiserum against CLCuV virions were tested for the detection of CLCuV through ELISA method. A dilution of 1:10 in case of coating buffer, 1:300 in antiserum and 1:1000 of conjugate appeared to be optimum for the testing of antigen. The four antisera (referred above) were tested against diseased/ healthy samples of HS-6 variety and there were distinct differences in absorbencies of infected plants and controls when antisera raised against ACMV virions were tested. The antiserum raised here against CLCuV also appeared effective in detection of disease.*


## Introduction

Cotton Leaf Curl Virus (CLCuV) disease has assumed serious proportions in the irrigated cotton belt of north India comprising an area of fifteen lakh hectares. The disease caused by a white fly transmitted Gemini virus was first noticed in Nigeria on *Gossypium peruvianum* and *G. vitifolia* (Farquharson 1912). In India the disease after its report in patches around Sriganganagar district of Rajasthan on *G. hirsutum* in 1993, spread to the entire north India in a short span of 4-5 years (Narula *et al*., 1999). The initiation of disease is characterized by small vein thickening (SVT) type symptoms on young upper leaves of plants. Upward leaf curling followed by formation of cup shaped leaf laminar out growths of veinal tissue on the abaxial side of the leaves, is other important symptom. In severe cases reduction of internodal length leading to stunting and reduced flowering/fruiting is also noted. The disease is known to be caused by DNA-A/DNA-1/DNA beta complex (Briddon *et al*., 2001). The effective management of this important disease is possible by development of resistant varieties, white fly control and eradication of weed hosts carrying this disease (Monga, *et al*., 2001 Raj *et al*., 2002). Identification of sources of resistance and development of detection system for the disease are important steps in this direction. In the present studies, standardization of a screening methodology, identification of resistant lines and detection of CLCuV disease using antiserum has been attempted.

## Experimental procedure

### Germplasm screening

Germplasm lines screening was carried out under field conditions against CLCuV disease from 1997 to 2001 and between 1040 and 1531 lines were screened each year during the period. Twenty plants of each line were sown at 67.5 cm x 30 cm. The recommended package of agronomic practices was followed. One line of susceptible check was planted after every ten rows. Observations on the incidence of leaf curl were recorded at peak disease period by counting the number of CLCuV affected plants and working out the percent disease incidence. Data on white fly population was also recorded from three plants by taking three (top, middle and bottom) leaves from each plant.

### Development of screening nursery

In order to develop a screening nursery, a one-acre field was selected on the north-eastern boundary of the station based on wind direction for most part of the disease progress period. Two rows of leaf curl susceptible variety F-846 and one row of white fly susceptible variety HS-6 were planted on three sides of this field so as to create a hedge for the purpose of CLCuV screening. This hedge with CLCuV infected plants was pruned in the off-season to maintain the inoculum of the disease. The susceptible variety HS-6 was sown in 150 $m^2$ area and progress of disease was monitored in this nursery. Biweekly observations on CLCuV and white fly incidence were recorded.

### CLCuV detection

The following antisera (i) ACMV coat protein fusion protein antisera,(ii) Antiserum raised against purified ACMV virions (iii) Antiserum against CLCuV virions received from R.W. Briddon were tested for the detection of CLCuV through ELISA method. An experiment was conducted to standardize the concentrations of coating buffer, antiserum and conjugate by taking their varying concentrations. The detection of CLCuV in the susceptible variety HS-6 was attempted with these antisera through ELISA technique. The detection was

also tried with antiserum raised at the station for which following procedure was adopted:

1. Crude antigen (CLCuV) was extracted from cotton leaf curl virus infected *G. hirsutum* var. HS-6 leaves of cotton plant in sodium carbonate buffer (pH 9.6) containing 0.01M sodium diethyl dithiocarbonate (Na-DIECA) and 2% polyvinyl pyrrolidone (PVP) and centrifuged at 10,000rpm for 20 minutes.
2. Diluted concentration (1/10 time) of crude antigen was used for immunization of two rabbits (*Rab.1 and Rab.2)* with suitable adjuvant on different time intervals, i.e. first dose on 0 days, Second dose on 20 days, third dose on 34 days (all subcutaneous) and last dose on 40 days was administrated intravenously without adjuvant.
3. Titer of primary antibodies was tested with control before giving booster dose with crude diluted antigen by DAC-ELISA
4. One week after booster dose, rabbits were bled for antiserum extraction. Antiserum was separated by centrifugation.

## Results and Discussion

### Germplasm screening

Among the 1040 to 1531 lines that were screened each year from 1997 to 2001between 170 and 744 were identified as free in each of the five years of screening. The lines found free in one year were not necessarily free during the next year also. Leaf curl incidence on affected lines ranged from 0 to 100 percent. White fly incidence ranged from 0.07 to 14.5/leaf (Table 1). Sixty-five lines that remained disease-fee over the five-year period of screening were identified and yield parameters of few promising lines are given (Table 2). The seed cotton yield/plant varied from 54 to 216 grams/plant. The boll weight and boll number ranged from 3.0-3.9 gram and 15.5 to 51 respectively. GOT up to 39.1 was noted in these lines. A number of entries resistant to leaf curl disease have been identified (Singh *et al*., 2000, 2001).

### Development of screening nursery

The appearance of disease in screening nursery was noted on 25$^{th}$ June on variety HS-6 in 2001. The incidence of CLCuV was 3% on 27$^{th}$ week. The maximum increase of disease was noted in the month of July and 76% incidence of leaf curl disease was observed on 1$^{st}$ August. Thereafter the progress of disease became slow in the month of August and September and a maximum incidence of 91% was noted on 38$^{th}$ week. The population of whitefly remained low and started rising in August end and September and a maximum population of 2.1/leaf was recorded (Table 3). The disease progress was also plotted on graph to study the development of infection loci and spread of disease. It was noted that the disease spread from initial few points and covered the entire area in the month of July. The black dots on the graph show fresh disease on each date whereas red dots show previous dates cumulative disease (Figure 1). Similarly in 2002 the maximum increase of disease was noted in the month of July when it increased from 15% to 53%. Thereafter the disease progress slowed down and the maximum incidence of 72% was recorded in 37$^{th}$ week. A maximum of 1.02 whitefly/leaf was recorded in 37$^{th}$ week (Table 4). Observations on the progress and incidence in the adjoining field were also recorded and it was noted that the maximum incidence was 12% and 14% on the same variety HS-6 in 2001 and 2002 respectively (Table 5). The material received from All India Coordinated Cotton Improvement Project was successfully screened in the field as shown by one example in Table 6.

### CLCuV detection

The data revealed that a dilution of 1:10 in case of coating buffer, 1:300 in antiserum and 1:1000 of conjugate appeared to be optimum for the testing of antigen as reduction in absorbance was noted with further dilutions (Table 7). The testing of CLCuV antiserum was tried in variety HS-6 where slight differences in absorbance in diseased and healthy samples were observed (Table 8). In another experiment, the antisera (ACMV, ACMV FP-5 and ACMV FP-6)were tested against diseased/healthy samples of HS-6 variety where antisera at 1:250 dilution and conjugate at 1:750 dilution were taken. There were distinct differences in absorbencies of infected plants and controls when antiserum raised against ACMV virions was tried (Table 9). The detection was also attempted from antiserum raised against partially purified CLCuV antigen and good differences in absorbance were noted (Table 10). A stock culture of CLCuV from Pakistan was readily detected in triple sandwich ELISA by antibodies raised against the particles of three other gemini viruses *viz*., *African Cassava Mosaic*, *Indian Cassava Mosaic* and *Okra Leaf Curl* (Harrison *et al*., 1997). In another study CLCuV reacted with two out of 17 monoclonal antibodies (Mabs) raised to *African Cassava Mosaic Virus* (ACMV) and five out of 10 Mabs raised to *Indian Cassava Mosaic Virus* (ICMV) (Nateshan *et al*., 1996).

The present studies have revealed the existence of sources of resistance against CLCuV disease. The screening nursery for this important disease has been standardized and the disease can be successfully detected using ELISA technique. These studies have contributed substantially to our understanding of the epidemiology and management of this disease.

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***Table 1.*** *Screening of germplasm lines against CLCuV disease.*

| Year | Lines tested | Lines free | Lines showing incidence | Range of disease incidence | Range of white fly/leaf |
|---|---|---|---|---|---|
| 1997 | 1267 | 744 | 523 | 3.85-93.75 | 0.07-1.80 |
| 1998 | 1220 | 726 | 494 | 3.85-80.00 | 0.07-1.27 |
| 1999 | 1531 | 326 | 1205 | 5.88-100.00 | 0.07-1.13 |
| 2000 | 1040 | 354 | 686 | 7.69-71.40 | 0.17-7.5 |
| 2001 | 1233 | 170 | 1063 | 11.11-100 | 0.16-14.5 |

***Table 2.*** *Yield parameters of few CLCuV free lines.*

| Sr. no | Entry | SCY/plant | Boll weight (g) | Boll no. | GOT % |
|---|---|---|---|---|---|
| 1 | SA 1267 | 87.0 | 3.2 | 51 | 32.1 |
| 2 | SA 1274 | 90.0 | 3.9 | 35 | 32.9 |
| 3 | SA 1299 | 86.0 | 3.8 | 33 | 33.7 |
| 4 | SA 1300 | 75.0 | 3.8 | 43 | 36.1 |
| 5 | SA 1331 | 54.0 | 3.2 | 15.5 | 38.9 |
| 6 | SA 1345 | 83.0 | 3.0 | 26.3 | 34.5 |
| 7 | SA 1346 | 114.0 | 3.3 | 44 | 33.2 |
| 8 | SA 1505 | 90.3 | 3.2 | 30 | 31.2 |
| 9 | SA 1263 A | 140.0 | 3.8 | 35 | 39.1 |
| 10 | SA 1278 | 216.0 | 3.9 | 37 | 33.1 |

***Table 3.*** *Progress of leaf curl disease in screening nursery (2001).*

| Week no | Percent CLCuV | White fly/leaf |
|---|---|---|
| 27 | 3.11 | 0.23 |
| 29 | 31.71 | 0.71 |
| 31 | 76.45 | 0.85 |
| 32 | 81.12 | 0.89 |
| 34 | 87.93 | 0.98 |
| 35 | 89.68 | 1.6 |
| 38 | 90.66 | 2.1 |

***Table 4.*** *Progress of CLCuV in screening nursery (2002).*

| Month | Week No. | CLCuV % | White Fly/ Leaf |
|---|---|---|---|
| Jun 2002 | 23 | 1.33 | 0.00 |
| | 24 | 2.45 | 0.02 |
| | 25 | 3.52 | 0.05 |
| July 2002 | 26 | 15.13 | 0.03 |
| | 27 | 21.43 | 0.05 |
| | 28 | 28.20 | 0.17 |
| | 29 | 42.13 | 0.22 |
| | 30 | 53.20 | 0.53 |
| Aug 2002 | 31 | 57.28 | 0.37 |
| | 32 | 59.20 | 0.42 |
| | 33 | 62.78 | 0.35 |
| | 34 | 65.11 | 0.32 |
| Sep 2002 | 35 | 67.83 | 0.42 |
| | 36 | 59.11 | 1.00 |
| | 37 | 71.83 | 1.02 |
| CD at 5% | | 3.94 | 0.28 |

***Table 5.*** *Progress of CLCuV incidence in adjoining field.*

| Week no | Leaf curl % | Week no | Leaf curl % |
|---|---|---|---|
| 27 | 0.0 | 24 | 0.0 |
| 29 | 3.61 | 28 | 0.0 |
| 32 | 6.07 | 30 | 3.71 |
| 34 | 10.53 | 32 | 7.13 |
| 37 | 10.63 | 34 | 7.41 |
| 39 | 10.74 | 36 | 12.62 |
| 42 | 11.74 | 38 | 13.96 |
| CD at 5% | NS | | NS |

***Table 6.*** *Screening of entries against CLCuV Br05(b)1.*

| S.No | Entry No. | ClCuV (%) | ClCuV Grade | Seed cotton yield (q/ha) |
|---|---|---|---|---|
| 1 | 451 | 0.0 | 0.0 | 1.90 |
| 2 | 452 | 0.0 | 0.0 | 0.13 |
| 3 | 453 | 8.8 | 1.0 | .87 |
| 4 | 454 | 0.0 | 0.0 | 1.15 |
| 5 | 455 | 43.0 | 3.33 | - |
| 6 | 456 | 0.0 | 0.0 | .36 |
| 7 | 457 | 61.7 | 4.0 | .12 |
| 8 | 458 | 73.3 | 3.67 | .12 |
| 9 | 459 | 0.0 | 0.0 | .25 |
| 10 | 460 | 16.4 | 1.67 | .20 |
| 11 | 461 | 8.3 | 1.0 | .056 |
| 12 | 462 | 0.0 | 0.0 | 2.08 |
| 13 | 463 | 0.0 | 0.0 | 1.05 |
| 14 | 464 | 0.0 | 0.0 | .37 |
| 15 | 465 | 13.3 | 1.67 | 3.35 |
| 16 | 466 | 13.3 | 1.0 | 1.47 |
| 17 | 467 | 8.3 | 1.0 | .19 |
| 18 | 468 | 0.0 | 0.0 | 1.33 |
| 19 | 469 | 24.4 | 2.33 | 1.05 |
| 20 | 470 | 11.1 | 1.0 | .51 |
| 21 | 471 | 5.6 | 1.0 | 1.68 |
| 22 | 472 | 0.0 | 0.0 | .19 |
| CD at 5% | | 21.0 | - | 1.37 |

***Table 7.*** *Standardization of coating buffer, antiserum and conjugate concentrations***.*

| ** | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.178 | 0.138 | 0.163 | 0.122 | 0.098 | 0.080 | 0.107 | 0.131 | 0.100 | 0.096 | 0.103 | 0.108 |
| B | 0.150 | 0.096 | 0.079 | 0.077 | 0.069 | 0.074 | 0.087 | 0.093 | 0.082 | 0.114 | 0.104 | 0.092 |
| C | 0.114 | 0.082 | 0.059 | 0.061 | 0.061 | 0.061 | 0.059 | 0.064 | 0.067 | 0.076 | 0.082 | 0.087 |
| D | 0.129 | 0.063 | 0.064 | 0.059 | 0.057 | 0.057 | 0.064 | 0.076 | 0.079 | 0.072 | 0.077 | 0.090 |
| E | 0.084 | 0.086 | 0.064 | 0.063 | 0.073 | 0.086 | 0.071 | 0.081 | 0.071 | 0.075 | 0.096 | 0.116 |
| F | 0.097 | 0.089 | 0.066 | 0.063 | 0.063 | 0.069 | 0.066 | 0.063 | 0.064 | 0.070 | 0.081 | 0.081 |
| G | 0.100 | 0.093 | 0.081 | 0.068 | 0.075 | 0.073 | 0.092 | 0.079 | 0.093 | 0.085 | 0.098 | 0.110 |
| H | 0.102 | 0.090 | 0.074 | 0.071 | 0.083 | 0.096 | 0.087 | 0.091 | 0.100 | 0.094 | 0.103 | 0.100 |

* 1/10, 1/20, 1/40, 1/80, 1/160 & 1/320 time dilutions of coating buffer

** 1/300, 1/600, 1/1200 &1/ 2400 dilutions of antiserum

*** Conjugate dilution 1 to 6 & A to D 1/1000
7 to 12 & A to D 1/2000
1 to 6 & E to H 1/3000
7 to12 & E to H 1/4000

***Table 8.*** *Absorbencies of samples extracted from variety HS-6 and tested against CLCuV antiserum.*

| | *Infected* | | | | | | Uninfected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 0.499 | 0.366 | 0.379 | 0.322 | 0.312 | 0.299 | 0.238 | 0.283 | 0.294 | 0.294 | 0.294 | 0.341 |
| B | 0.441 | 0.332 | 0.274 | 0.259 | 0.239 | 0.237 | 0.188 | 0.224 | 0.236 | 0.236 | 0.272 | 0.305 |
| C | 0.371 | 0.255 | 0.216 | 0.217 | 0.223 | 0.206 | 0.169 | 0.178 | 0.200 | 0.200 | 0.203 | 0.239 |
| D | 0.312 | 0.229 | 0.202 | 0.234 | 0.204 | 0.204 | 0.172 | 0.162 | 0.167 | 0.167 | 0.181 | 0.247 |
| E | 0.213 | 0.156 | 0.157 | 0.168 | 0.154 | 0.145 | 0.126 | 0.128 | 0.126 | 0.126 | 0.142 | 0.192 |
| F | 0.212 | 0.170 | 0.149 | 0.141 | 0.151 | 0.134 | 0.122 | 0.121 | 0.127 | 0.127 | 0.124 | 0.164 |
| G | 0.188 | 0.176 | 0.162 | 0.152 | 0.197 | 0.140 | 0.126 | 0.136 | 0.119 | 0.119 | 0.127 | 0.156 |
| H | 0.266 | 0.176 | 0.241 | 0.164 | 0.187 | 0.179 | 0.129 | 0.165 | 0.140 | 0.140 | 0.150 | 0.173 |

Coating buffer 1/10; Antiserum 1/500

| | | |
|---|---|---|
| Conjugate dilution | 1 to12 & A to D 1/1000; 1 to 12 & E to H 1/2000 | |
| Infected | 1 to 6 & A to D 1/1000 | 0.284 |
| Uninfected | 7 to 12 & A to D 1/1000 | 0.225 |
| Infected | 1 to 6 & E to H 1/2000 | 0.174 |
| Uninfected | 7 to12 & E to H 1/2000 | 0.137 |

***Table 9.*** *Absorbances of samples extracted from variety HS-6 and tested against ACMV antisera.*

| Antiserum | Infected | Healthy |
|---|---|---|
| ACMV | 0.724 | 0.373 |
| ACMV FP-5 | 0.869 | 0.769 |
| ACMV FP-6 | 0.805 | 0.615 |

***Table 10.*** *Absorbances of diseased and healthy samples tested by antiserum raised against partially purified CLCuV antigen.*

| | *Rab.1* | | *Rab.2* | |
|---|---|---|---|---|
| Titer of antiserum | 1:250 | 1:500 | 1:250 | 1:500 |
| Healthy | 1.322 | 1.072 | 0.859 | 0.655 |
| Diseased | 1.822 | 1.146 | 1.895 | 1.600 |

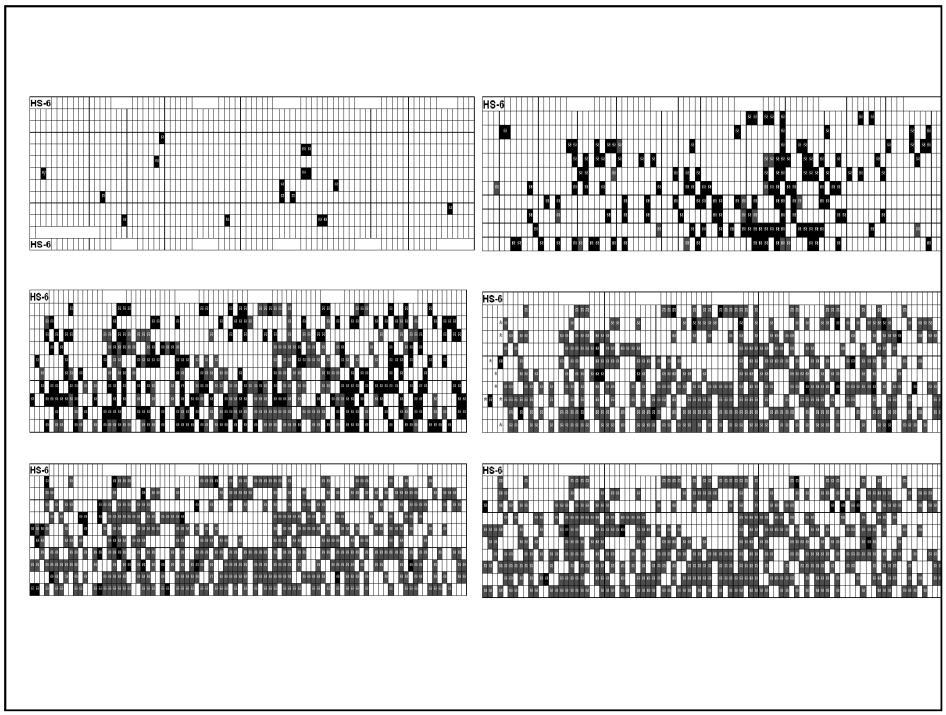


***Figure 1.*** *Fortnightly progress of CLCuV disease in screening nursery (5th and 21st Jul, 1st, 10th, 21st and 30th Aug) during the 2001season.*