1517 Recent research advances to combat cotton leaf curl virus (CLCUV) disease in Pakistan

Dr. Zahoor Ahmad Baluch , Pakistan Central Cotton Committee, Karachi, Pakistan

ABSTRACT

Studies were undertaken to develop management strategy to combat CLCuV disease. The experiments included intra- and inter-specific hybridization programme, cultural and nutritional management measures. The results of the studies have shown that transfer of resistant gene from wild species to Upland cottons have shown promise to control this disease. The material is at advanced stage for commercialization purpose. The disease could also be managed by manipulating crop maximization practices. The planting of cotton after 15th May to 15th June would result in reducing the severity of the disease. Application of potassium fertilizer @ 100 kg K_2O ha^{-1} with combination of 150 kg N ha^{-1} helps in the reduction of CLCuV disease by about 20-30 percent. There is great variability in the natural incidence of disease in various localities in the cotton belt of the Punjab province.

Keywords: *Gossypium hirsutum* L., Cotton Leaf Curl Virus Disease, Inter-specific Hybridization, Petiole Graft Transmission Technique, Nutritional Management, Cultural Management.

INTRODUCTION

Cotton is lifeline of the country. It is an occupation of more than 1.5 million farming families and a source of livelihood for several million of labour in the cities and towns. In cotton growing areas, the sale of cotton produce may account as much as 40% of cash income of rural household. Besides this, it accounts for 60% of our export earnings and about 85% of domestic oil production. Apart from the main cotton growing areas in the Punjab and Sindh provinces, Balochistan province has emerged as potential area for cotton production. At present, in Balochistan cotton was sown on 40,000 hectares during 2006-07 and is likely to increase many-fold after the completion of water reservoirs. In terms of volume, there has however been a phenomenal growth in the cotton and textile sector of Pakistan over the past 57 years, which can well be adjudged from the simple fact that the number of textile mills in the country increased from only two in 1947 to 458 in 2005. This could obviously be possible due to increasing cotton production from merely 1.1 million bales in 1947-48 to an all time record crop of 13.0 million bales in 2005-06 (Fig.1).

Cotton leaf curl virus (CLCuV) disease was first reported during 1967 near Multan (Hussain and Ali, 1975). It is a viral disease transmitted by Whitefly (*Bemisia tabaci* and *B. argentifolii*), the patent insect vector. This disease belongs to the genus Begomovirus (family geminiviridae), Gemini virus subgroup III (Hameed *et al*., 1994). This disease is characterized by an upward curling of leaves, thickening of veins and laminar outgrowth on underside of the leaf called 'enation' (Mahmood, 1999; Khalid *et al*., 1999; Akhtar *et al*., 2002a). During the year 1991-92, Pakistan achieved a record production of 12.82 million bales. During the year 1992-93, CLCuV disease appeared in epidemic form which caused decrease in cotton yield down to 9.05 million bales and further reduced to 7.9 million bales during 1994-95

(Anonymous 1997). Since then, the yield losses have become a constant phenomenon every year due to this disease. A new strain of CLCuV was diagnosed in Burewala territory of the Punjab in 2001-02 which is more virulent than previous strain of virus. Various researchers reported that this new strain is a new version of current CLCuV. The re-emergence of CLCuV was named the Burewala Strain of Cotton Virus (BSCV) after the place where it was first detected. This virulent mutated version also attacked the parent material (LRA-5166, CP-15/2 and Cedex), which had been used earlier to develop CLCuV resistant varieties (Tahir and Mahmood, 2005). BSCV has infested the most productive areas in the central Punjab (Khanewal, Multan, Lodhran, Vehari, Bahawalnagar, Bahawalpur) and is expected to lower production significantly in the Punjab in 2007-08. It is feared that BSCV may also spread to Sindh province. All the varieties which were resistant to CLCuV-Multan are prone to this new strain (Mahmood *et al*., 2003; Tahir *et al*., 2004). This strain of disease is termed as CLCuV-Burewala. This new strain is spreading widely across the cotton belt of Pakistan and posing a major threat to cotton production (Mahmood *et al*., 2003). The infection of the disease is more virulent at early stages of growth compared to the maturity (Brown and Bird, 1992 and Arshad *et al*., 2006). Various researchers have reported that this virus is neither mechanically transmissible nor carried in soil or seed (Ali *et al*., 1995). Transmission through petiole grafting and whitefly has been successfully done in Pakistan (Mirza, 1992 and Tahir *et al*., 1994). The control of CLCuV in the country could only be managed through development of resistant varieties and cultural management. Therefore, comprehensive studies are underway to combat this disease. The research in this regard were related to screening of genetic stock of cotton germplasm, intra- and inter-specific hybridization, testing of adaptability trials under various ecological zones, cultural and nutritional management were carried out.

In view of the economic losses attributable to this viral disease, Government of Pakistan is financing a number of research projects in the country to conduct research on this disease (Anonymous, 2003).

MATERIALS AND METHODS

Studies were carried out under field and greenhouse conditions at various locations in the Punjab and Sindh province. Survey was conducted to assess the incidence of BSCV in various districts of the Punjab. The fields were randomly surveyed and four spots of 100 plants were randomly selected from each union. Incidence was then determined by counting the healthy and disease plants (Anonymous, 1994) and incidence of CLCuV was estimated at the level of mild (infection upto 20%), medium (infection upto 40%), and severe infection (infection upto 80%) [Tahir and Mahmood, 2005). The germplasm was screened to determine its tolerance level to BSCV according to Ali *et al*., 1995. The intra- and inter-specific hybridization material was screened to determine its reaction to BSCV disease. Data on epidemiological studies were recorded from multidimensional experimentation laid out at Central Cotton Research Institute, Multan.

RESULTS

Data on natural incidence on BSCV disease in Multan, Khanewal, Vehari, Lodhran, Bahawalpur, Muzafargarh, Burewala revealed that all the varieties cultivated in these areas were susceptible to BSCV (Table-1). The natural incidence ranged from 12.13 to 40.45. The worst hit area was Burewala followed by Vehari. The variation in the

natural incidence may be accrued due to presence of incoculum of different degree in these places.

The commercial varieties and un-recommended cultivars differed greatly in their response to CLCuV disease in the cotton belt of the Punjab. Cultivar BH-163, CIM-511, VH-144 were worst affected by BSCV disease. In terms of mean losses, BH-163 was worst affected compared to least affected NIAB-222 (Table-2).

Data presented in Table-3 showed that genetic stock developed through hybridization at the Central Cotton Research Institute, Multan was highly susceptible to BSCV disease. The material was tested through petiole graft transmission technique.

Strains included in the National Coordinated Varietal Trial (NCVT) planted at Multan and Vehari sites were also susceptible to BSCV disease. The reaction of disease to various varieties differed at both locations (Table-4). The incidence of disease in strain CIM-538 was 56.63 % at Multan whereas it was 20.70% at Vehari. On the other hand, strain TH-84/99 had 28.13% at Multan compared to 60.4% at Vehari.

The strains included in the NCV trial were further tested for their reaction to BSCV disease through petiole-graft transmission. Data showed that all the strains had a rating of 5 and 6 i.e. severely affected by this viral infection (Table-5). The number of days taken to appear symptoms of the disease differed greatly in various strains. The strain TH-41/83 and MNH-700 took less number of days (7-9) compared to FH-115 and CIM-496 taking more number of days (21-28).

Data presented in Table-6 showed that material developed through inter-specific hybridization had greater tolerance to the disease. The self seed of one plant (*G. hirsutum x G. anamolum*) showed resistance against disease. The progenies of these individual plants are being tested for their resistance to the disease.

Data presented in Table-7 showed that some elite plants showing resistance to BSCV disease have comparable fibre quality parameters to that of other commercial varieties. Plant number CP-11 has fibre length of 33 mm with concurrent fibre strength of 119.8 tppsi.

The incidence of BSCV disease was greatly influenced by planting dates (Table-8). The crop planted on 15th May and 1st June was greatly hit by the disease compared to 15th and 30th June planted crop. Crop planted on 15th May had 60.56 percent compared to 34.70 percent in May 1 planted crop.

Application of nitrogen and potassium fertilizers produced beneficial effects in reducing the incidence of disease (Table-9). Addition of 150 kg N ha^{-1} and 100 kg K_2O ha^{-1} caused reduction of about 50 percent compared to untreated check. The values of disease varied from 7.5 to 15 percent in various treatments.

Incidence of disease was greatly affected by plant to plant spacing (Table-10). The narrowing of distance from plant to plant caused decrease of incidence in disease compared to at wider spaces between plants. The incidence of disease increased from 8cm to 23cm by increasing the distance from plant to plant.

The fibre quality was greatly affected by BSCV disease. The plants severely affected by the disease had lower fibre quality compared to that of healthy ones (Table-11).

DISCUSSION

The variability in the natural incidence of disease depends upon the genetic makeup of the cultivar, concentration of inoculum of the disease and cultural management at different sites. Furthermore, the pressure of whitefly with concurrent presence of inoculum in the area affects the incidence of the disease. The results of this study corroborates with those of Tahir and Mahmood (2005). They reported that natural incidence of the disease varied from 14.4 to 70.24 % in various districts of the Punjab during 2004-05. Similarly the varieties planted in these districts had great variability in their reaction to the disease. The similar results have been reported by Tahir *et al*., 2005 and Mahmood *et al*., 2003.

The genetic stock maintained by different research institution of Pakistan have narrow genetic base. It is highly susceptible to BSCV disease. Various researchers (Mahmood, 1999; Mahmood *et al*., 2002; Tahir and Mahmood, 2005) reported that the existing germplasm is susceptible to BSCV disease. The introduction of exotic material could help in widening the genetic base for inclusion of resistance to BSCV disease in Upland cottons. The variability in different strains reaction to BSCV disease is dependent upon its genetic makeup and presence of suitable environment.

The material developed through inter-specific hybridization for development of BSCV resistant strains was tested through petiole-graft transmission. The material showed prospects in delaying the appearance of symptoms in the material. This material had varying degrees of appearance of disease. Much advances have been made in developing resistance by transferring of virus resistant gene from wild species into Upland cottons (Naveed and Anjum, 2007).

The management strategy can be adopted to avoid the severity of the disease. Different researchers (Ali *et al*., 1995; Mahmood, *et al*. 2001; Tahir *et al*., 2004; Arshad *et al*., 2006) reported that late planting of cotton had lower incidence of disease than earlier sowing of cotton. Furthermore, virus incidence decreased with increasing levels of nitrogen and potassium applied fertilizers separately or in combination. The combined application of nitrogen and potassium fertilizers had a significant impact in reducing BSCV incidence. The fibre quality is affected by the health of the plant. The poor translocation of nutrients from source to sink and reduction in growth results in production of poor quality cottons (Makhdum, 2004).

ACKNOWLEDGEMENTS

I am highly obliged of Mr. Muhammad Arshad, Director, Central Cotton Research Institute, Multan Pakistan for write up and technical support. I also extend my thanks to Dr. Muhammad Iqbal Makhdum, Consultant, Central Cotton Research Institute, Multan for peer reviewing the paper.

REFERENCES

Akhtar, K.P., M.A. Haq; M. Hussain; Al. Khan. 2002a. Whitefly transmitted Gemini virus and associated disorder in cotton: a review. Pak. J. Phytopath. 14: 140-150.

Ali, M.; Ahmad, Z.; Tanveer, M. and Mahmood, T. 1995. Cotton leaf curl virus in the Punjab: current situation and review of work. Central Cotton Research Institute, Multan. 117 p.

Anonymous, 1994. Methods of recording observations. In "Programme of work of Pest Warning Directorate for the year 1994 regarding the control of cotton leaf curl virus". Directorate of Pest Warning & Quality Control of Pesticides, Punjab.

Anonymous, 1997. Economic Survey. Government of Pakistan, Finance Division, Islamabad, Pakistan, 1996-97.

Anonymous, 2003. Cotton World Statistics, Bulletin of International Cotton Advisory Committee, Published by the International Cotton Advisory Committee, Washington, D.C., USA.

Arshad M., Zafar, Y., and Noor ul Islam. 2006. New virus threatens cotton production in Pakistan. ICAC Recorder, 13-18.

Brown, J.K. and Bird, I. 1992. Whitefly transmission geminivirus in the Americas and the Caribbean basin: past and present. Plant Diseases. 76:220-228.

Hameed, S., S. Khalid, Ehsan ul Haq, and A.A. Hashmi. 1994. Cotton leaf curl disease in Pakistan by a whitefly transmitted geminivirus. Plant Diseases 78: 528.

Hussain, T. and Ali, M. 1975. A review of cotton diseases of Pakistan. The Pakistan Cottons. 19:71-86.

Khalid, S., H. Shah and M.A. Masood. 1999. Relationship of cotton leaf curl virus symptoms with virus concentration and epitope profile. Pak. J. Biol. Sci., 2: 1387-89.

Mahmood, T. 1999. Cotton leaf curl virus disease and its status in Pakistan. Proceedings of ICAC-CCRI Regional Consultation on Insecticide Resistance Management in Cotton. pp 234-244.

Mahmood, T.; Nadeem, A. and Nelson, M.R. 2001. Impact of seed treatment with systemic insecticides on cotton leaf curl virus disease. Pak. J. Biol. Sci. 4: 146-148.

Mahmood, T.; Tahir, M.; Mahmood, H.T. and Hussain, S. 2002. Testing of cotton leaf curl virus resistant of candidate varieties/strains through different techniques. Asian J. Plant. Sci. 1: 489-491.

Mahmood, T.; Arshad, M.; Gill, M.I.; Mahmood, H.T.; Tahir, M. and Hussain, S. 2003. Burewala strain of cotton leaf curl virus: a threat to CLCuV cotton resistant varieties. Asian J. Plant Sci. 2: 968-970.

Makhdum, M.I. 2004. Response of some cultivars to sulphate of potash and muriate of potash. PhD dissertation. Bahauddin Zakariya University, Multan.

Mirza, M.S. 1992. Virus problem in cotton and its control. Proceedings of National Seminar on Cotton Production at Ayub Agricultural Research Institute, Faisalabad on April 30, 1992.

Naveed, M. and Anjum Z.I. 2007. Resistance found for Burewala Strain Cotton Virus (BSCV). International & European whitefly studies network website Jan, 2007.

Tahir, M. and Mahmood, T. 2005. Occurrence of cotton leaf curl virus in the main cotton belt of the Punjab and its impact on yield losses during 2004-05. The Pakistan Cottons, 49: 65-73.

Tahir, M., Naveed, M., and Mahmood, T. 1994. Varietal response to leaf curl virus on early sown cultivars of cotton. Pak. J. Phytopathol. 6: 107-109.

Tahir, M.; Mahmood, T.; Mahmood, H.T. and Hussain, S. 2004. Effect of sowing dates on incidence of cotton leaf curl virus disease on different cultivars of cotton. Plant. Path. J. 3:61-64.

Tahir, M.; Mahmood, T; Mahmood, H.T. and Hussain, S. 2005. Infestation of cotton plant by cotton leaf curl virus disease as induced by plant senescence, whitefly dynamics and its resistance capacity. The Pakistan Cottons, 49: 13-19.

Fig. 1: Cotton Area, Production & Yield in Pakistan

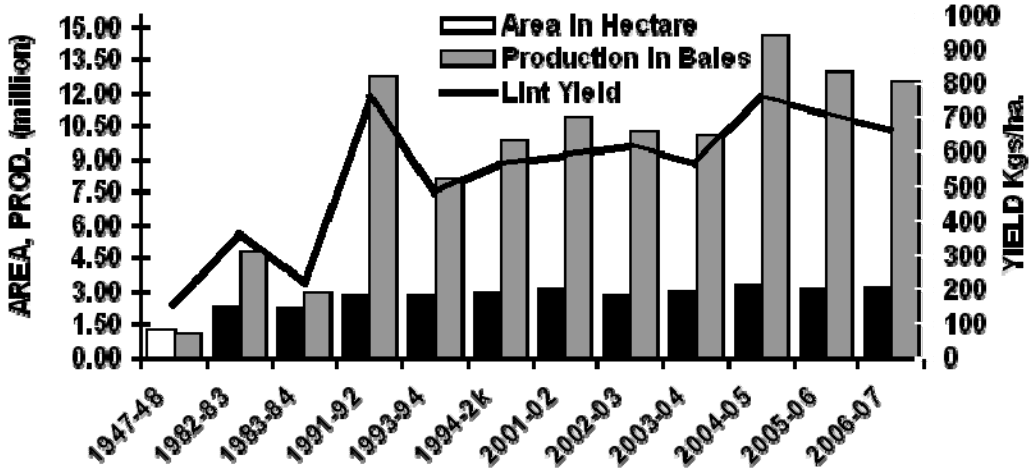

Table-1 Natural incidence of CLCuV (BSCV) at different sites of the Punjab province

District	No. of Sites	Area (acres)	CLCuV Incidence (%)				Mean losses
			A	B	C	Total	
Multan	19	211	18.27	11.85	3.02	33.14	10.81
Khanewal	18	175.5	7.09	4.04	1.00	12.13	3.83
Vehari	13	112	19.86	13.57	5.18	38.61	13.54
Lodhran	9	173	14.11	8.63	0.92	23.66	7.01
Bahawalpur	10	215	21.47	12.90	3.15	37.52	11.97
Muzafargarh	7	65	7.66	4.94	0.72	13.32	4.08
Burewala	12	81	20.92	14.46	5.07	40.45	14.02

A = Mild infection or loss upto 20% B = Medium infection or loss upto 40%

C = Severe infection or loss upto 80%

Table-2 Incidence of CLCuV and Mean Losses in different cultivars in the Punjab province.

Variety	Frequency	Area (acres)	CLCuV Incidence %				Mean Loss %
			A	B	C	Total	
BH-95	1	15	15.00	10.00	2.00	27.00	8.60
BH-118	1	3	5.00	2.00	0.00	7.00	1.80
BH-142	1	10	13.00	9.00	2.00	24.00	7.80
BH-160	28	216	14.93	9.90	2.73	27.56	9.13
BH-162	2	9	15.00	8.00	2.00	25.00	7.80
BH-163	1	16	45.00	25.00	8.00	78.00	25.40
CIM-446	7	45	12.13	7.60	1.60	21.33	6.74
CIM-473	14	77	22.92	7.33	1.88	32.13	9.02
CIM-496	69	664	13.78	9.41	2.49	25.68	8.51
CIM-497	1	4	8.00	4.00	0.00	12.00	3.20
CIM-499	16	132	16.25	11.17	4.21	31.63	11.07
CIM-506	32	265	13.84	9.76	2.37	25.97	8.56
CIM-511	2	10	26.00	16.50	1.50	44.00	13.00
CIM-534	1	8	12.00	8.00	1.00	21.00	6.40
FH-945	1	2	15.00	4.00	0.00	19.00	4.60
FH-1000	1	4	4.00	2.00	0.00	6.00	1.60
MNH-526	1	28	13.00	8.00	2.00	23.00	7.40
MNH-584	1	3	8.00	2.00	0.00	10.00	2.40
MNH-700	1	5	4.00	3.00	0.00	7.00	2.00
NIAB-98	1	3	3.00	1.00	0.00	4.00	1.00
NIAB-111	17	156.5	12.80	8.51	2.06	23.37	7.61
NIAB-222	1	3	5.00	1.00	0.00	6.00	1.40
NIAB-884	3	19	7.66	4.00	0.00	11.66	3.13
VH-141	1	2	10.00	7.00	1.00	18.00	5.60
VH-142	12	71	17.08	11.50	3.05	31.67	10.45
VH-144	5	36	19.00	11.33	2.00	32.33	9.93
VH-148	1	5	3.00	2.00	1.00	6.00	2.20

A = Mild infection or loss upto 20%, B = Medium infection or loss upto 40%,

C = Sever infection or loss upto 80%

Table-3 Screening of genetic stock through petiole-graft transmission for reaction to BSCV

Experiment	No. of families screened	No. of families showing resistant to CLCV
Varietial Trial	69	0
Micro Varietal Trial	114	0

Table-4 Screening of strains included in National Coordinated Varietal Trial (NCVT) through petiole-graft transmission.

Strains	Sites	
	Multan	Vehari
BH-162	36.88	31.67
NIAB-884	28.96	26.94
MJ-7	23.20	13.91
TH 84/99	28.13	60.40
FH-115	31.07	24.09
NIAB-824	24.13	15.40
MNH-786	11.94	10.30
CIM-534	29.68	27.90
FH-207	13.27	15.48
CRIS-466	44.54	27.40
MNH-786	11.28	9.06
PB-899	28.86	27.50
H-151-F2	10.31	6.76
CRIS-461	17.44	14.30
TH-35/99	27.01	9.94
NIBGE-4	20.93	23.40
CIM-499	56.12	33.23
GH-99	33.29	44.83
CIM-538	56.63	20.70

Table-5 Screening of strains included in NCVT against CLCuV through petiole-graft transmission

Strains	**Reaction***	**No. of days taken to appear the symptoms (after grafting)**	**Intensity 0-6**
CIM-496	+++	21-26	5
CRIS-460	+++	17-19	6
FH-115	+++	22-28	6
NIAB-884	+++	14-29	6
MJ-7	+++	17-23	6
CIM-534	++	17-21	5
CRIS-461	+++	8-10	6
FH-2000	+++	16-20	6
NIAB-98	+++	7-11	6
H-151-F2	++	16-19	5
CIM-499	+++	14-18	6
TH-41/83	++	7-9	6
BH-162	++	8-13	5
PB-899	++	12-16	5
NEELUM NS-11	+++	13-16	6
SLH-279	+++	8-10	6
NIBGE-2	+++	14-17	6
MNH-700	+++	7-9	6

* - = No symptoms + = Mild ++= Medium +++ = Severe

Intensity Rating: 0 = Complete absence of symptoms; 1 = Few small scattered vein thickening; 2 = Small scattered vein thickening; 3 = Vein thickening involving small groups of veins; 4= Large groups of veins involved; 5 = All veins involved; 6 = All veins involved and severe curling

Table-6 Screening of interspecific material against CLCuV through petiole graft transmission

Parentage	No. of Plants Grafted	No. of plants under Observation	No. of plants showing symptoms	Number of days taken to appear symptoms	Severity
{^{2} hir x 2 (*G. hir* x *anom*.)} X CIM-702	160	10	150	10-26	Severe
{^{2} hir x 2 (*G. hir* x *anom*.)} X CIM-109	40	4	36	12-70	Severe
{^{2} hir x 2 (*G. hir* x *anom*.)} X Cyto-71	37	4	33	10-14	Medium
{^{2} hir x 2 (*G. hir* x *anom*.)} X Cyto-70	27	2	25	11-16	Medium
{^{2} hir x (*G.hir* x *anom*.)} X Cyto-71	75	21	54	12-120	Severe
{hir x 2 (*G. arbo* x *anom*.)} X Cyto-71	112	14	98	12-110	Medium
{^{2} hir x 2 (*G. arbo* x *thurberi*.)}	5	0	5	10-14	Medium
^{2} hir x 2 (*G. hir* x *anom)*	8	0	8	9-14	Severe
Total	**464**	**55**	**409**		

Table-7 Fibre characteristics of some elite plants showing resistance to BSCV disease

[{*G.* ^{3}*hirs*. x 2 (*G. hir*. x *G. anom*.)} x {*G.* ^{2}*hirs*. x 2(*arbo.* x *G. anom.*)}]

Plant No	Seed cotton yield (g plant^{-1})	Lint %age	Fibre length (mm)	Fibre micronaire (µg inch^{-1})	Maturity ratio (%)	Fibre strength (TPSSI*)
CP-3	31	34.5	30.0	3.3	0.89	102.4
CP-4	179	34.3	32.0	3.0	0.81	96.4
CP-6	85	39.1	31.8	4.2	1.02	106.7
CP-7	38	39.4	29.0	3.2	0.89	91.4
CP-11	28	32.6	33.0	3.1	0.85	119.8
CP-17	87	35.6	29.5	3.6	0.85	91.3

*thousand pounds per square inch

Table-8 Incidence of BSCV (%) as influenced by planting dates

Observation Dates	Planting Dates				
	1st May	15th May	1st June	15th June	30th June
10/6	0.62	-	-	-	-
26/6	13.77	10.50	-	-	-
10/7	34.70	60.56	58.99	-	-
26/7	61.61	91.77	91.79	35.91	-
10/8	61.61	91.77	91.79	54.30	35.09
26/8	72.28	91.77	93.98	61.13	62.33
10/9	72.28	92.37	93.98	63.73	74.98
26/9	72.28	92.37	93.98	63.73	75.08

Table-9 Interactive effects of nitrogen and potassium fertilizers on the incidence of BSCV (%).

Nutrient dose (kg ha^{-1})		Observation dates		
N	**K_2O**	**15th July**	**15th August**	**15th September**
0	0	8.0	12.7	15.0
	50	5.8	9.2	11.4
	100	4.0	6.5	8.5
100	0	4.7	10.7	12.8
	50	3.8	6.1	8.1
	100	3.2	5.2	6.7
150	0	4.6	7.4	10.2
	50	4.0	5.7	7.5
	100	3.7	5.7	7.5

Planting date = 15th May

Table-10 Incidence of BSCV disease (%) as affected by plant to plant spacing.

Plant-to-plant spacing	Observation dates							
	25/6	14/7	30/7	15/8	30/8	15/9	30/9	15/10
8 cm	2.08	8.22	10.82	20.77	35.49	35.49	35.49	35.49
15 cm	2.15	17.20	30.38	36.46	43.64	43.64	43.64	43.64
23 cm	3.14	41.73	60.52	66.92	75.59	81.88	82.50	82.50

Planting date = 15th May Row to row distance = 75 cm

Table-11 Effect of cotton leaf curl virus disease on fibre characteristics

Treatments	Fibre length (mm)	Uni-formity index	Micro-naire value (µg inch^{-1})	Maturity ratio	Fibre strength (tppsi)
Healthy plants	27.00	81.43	4.80	1.07	102.50
Virus effected plants	26.00	78.00	4.10	0.95	98.40