INTERSPECIFIC HYBRIDIZATION IN COTTON (*G.HIRSUTUM*) TO DEVELOP CLCUV TOLERANT VARIETIES

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ABSTRACT

Cytogenetics section is going on in genetic introgression work through interspecific crosses viz., *G arboreum* x *G anomalum,, G hirsutum* x *G anomalum*, and *G hirsutum* x *G stocksii*, to have CLCuV tolerance in *G. hirsutum.*. Pedigree method of selection from F2 to F8 with back crossing followed by selection was followed. Observations on CLCuV tolerance alongwith all desirable traits i.e. agronomic and fibre traits were recorded. Harland (1936) indicated the possibility of developing interspecific types with desired features of *G. barbadense* and *G. hirsutum* species. Rajarathinam and Nadarajan (1993), Rajarathinam *et al.* (1993); Murthy *et al.* (1995a), Murthy *et al.* (1995b) and Kumar *et al.* (2000) reported that there is maximum contribution of one or more yield components towards genetic divergence. 206 lines of F_4 $BC_2$of [{2(G. *hirs*. x *G.anom*.) x *G.* ^{3}*hirs*.} x {2(*G. arbo*.x *G.anom*.)x *G.* ^{2}*hirs*. }] were screened in field condition through out cotton season. The spreader was CIM 496 .After 45 days of sowing; CIM 496 showed 100% susceptibility against virus while seven lines were 100% free from CLCuV. From this resistant material Cyto-124 was developed and this line has lowest CLCuV %age and was on the top in Punjab, Pakistan in NCVT results. Transference of drought tolerance, salt tolerance and virus resistance from *G. stocksii* into *G. hirsutum* also a diamond source for CLCuV tolerance in cultivated cotton. Ahmad *et al.(*2011) found *G.stocksii* resistance to CLCuV. *G.hirsutum* x *G.stocksii* was crossed in 2006-07 and now at pentaploid stage. This will also provide a great resistance against CLCuV and drought tolerance. As a result of advances in the spinning technology of present day textile industries, higher cotton fibre quality, especially strength and length is a continuous process in Cytogenetics research programme which will help to fulfill the changing demands/needs of consumers. This is part of our research to improve Yield by developing CLCuV tolerance varieties with better fibre traits.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the backbone of Pakistan's economy accounts for 8.6% of the value added in agriculture and about 1.8% to GDP. Total area for cotton cultivation was 3.106 million hectares 10.1% more than last year 2.82 million hectares. Estimated production is 12.7million bales for 2009-2010, higher by 7.4% over last year production of 11.8million bales. Yield was 695Kg/hac. However cotton production was 5.0% less then the target of 13.36 million bales mainly due to spread of CLCV, shortage of irrigation water, high temperature in August resulting high fruit shedding and flare up of sucking pests (Agricultural Statistics of Pakistan 2009-2010).Cotton is infected by

several insects, pests and pathogens inducing different diseases. Among them Cotton Leaf Curl Virus is the most obnoxious disease causing enormous losses to the cotton crop (Khan & Ahmad, 2005). It has caused reduction of 9.45 million bales during the last decade, amounting to rupees 75 billion to the national economy. In 1981, the cotton area affected by this disease was about 35000 acres (Ali *et al.,* 1992). This disease spread in Sindh in 1997, in Khyber Pakhtoon Khawa in 1998 and in Baluchistan in 2001 (Tariq, 2005). Reduction in yield in tolerant varieties was 50% and 85-90% in susceptible cultivars (Hussain, 1995; Khan *et al*., 2001).

A new and mutant strain of CLCuV (Begomovirus) was observed in Burewala region 2001-02, which is more virulent and named the Burewala Strain of Cotton Virus (BSCV) after being detected in Burewala. In the wake of resurgence of new strain of Burewala virus the resistance was broken down and as a result all the varieties including LRA-5166, CP-15/2 and Cedex (resistant to previous CLCuV became susceptible to BSCV (Briddon, 2003; Mahmood *et al*., 2003; Tahir *et al*., 2004).

Different scientists worked on interspecific hybridization for transferring resistant genes for favorable traits from wild diploid species into tetraploid cultivated cotton like (Blank and Leathers, 1963) transferred resistant genes against cotton rust caused by *Puccinia cacabata.* from *Gossypium anomalum* L. and *Gossypium arboreum* L. into *Gossypium hirsutum* L. through interspecific hybridization, induction of polyploidy and back crossing accompanied by continuous screening for resistance. It is worthwhile to combine the genes for Cotton Leaf Curl Virus resistance and other diseases and drought resistance between *Gossypium hirsutum L.* and *Gossypium arboreum* L. cotton (Amin, 1940). Moreover other research workers have attained success in introgression of *Gossypium hirsutum L.* and *Gossypium arboreum* L. (Bao-Liang Z, Chen S, Xin-Lian S, Xiang-Gui, Z and Ahen-Lin A (2003). Moreover other research workers have attained success in interspecific introgression of *Gossypium hirsutum L.* and *Gossypium arboreum* L. (Bao-Liang *et al*., 2003).Similarly, (Knight 1957; Brinkerhoff, 1970) introgressed resistant genes B6 found in 'A' genome of *Gossypium arboreum* L. against bacterial blight caused by *Xanthomonas malvacearum*. Into *Gossypium barbadense* L. Breeders also achieved most resistant commercial variety 'Auburn 56'against Root knot nematode (*Meloidogyne incognita*) in U.S. cotton through transgressive segregation (Shepherd, 1974). Sacks and Robinson (2009) also introgressed resistance to *Rotylenchulusreniformis.* into the tetraploid 2 (AD1) through crossing a resistant diploid A2-genome *Gossypium arboreum* L. accession (A2-190) with a hexaploid 2 [(AD1) D4] bridging line (G 371) to obtain a tetraploid triple specie hybrid. At present no single variety of *Gossypium hirsutum* L. is resistant to BSCV, whereas *Gossypium arboreum* L. is known to have immunity against Cotton Leaf Curl Virus (Bird, 1973).Keeping in view losses made by CLCuV emphasis should be given to explore the possibility of successful transferring virus resistant genes from Desi cotton (*Gossypium arboreum* L. 2n=26) into cultivated Upland cotton (*Gossypium hirsutum* L. 2n = 52) genotypes through conventional breeding.

The Cytogenetics Section of Central Cotton Research Institute, Multan is engaged for the last many years in transferring desirable characters of wild species to the cultivated ones through complex crosses. While screening 30 Gossypium species in hand, it was observed that the diploid species of cotton viz. *G. herbaceum, G. arboreum, G. anomalum, G. captis viridis, G. gossypioides, G. laxum, G. stocksii, G. areysianum,*

G. somalense and *G. longicalyx* showed resistance to Burewala stain of cotton leaf curl virus.

MATERIALS AND METHODS

Cyto124, a new candidate strain which was developed through interspecific hybridization of [{2(G. *hirs*. x *G.anom*.) x *G.* ^{3}*hirs*. } x {2(*G. arbo*.x *G.anom*.)x *G.* ^{2}*hirs*. }] was reported a highly tolerant line in annual report of Central Cotton Research Institute, Multan during 2011-12. A pedigree method of plant breeding and selection was employed to advance the segregating populations. Following this method, 206 lines were screened. This material was sown on 19.05.2009 in plant to progeny row trial. The lay out was sick bed technique. Observations regarding CLCuV %age were taken fortnightly, after 30 days of sowing. The superior progenies were tested in a series of replicated trials before putting it into a national testing system The new variety was compared with a commercial variety CIM-496 as checks under national coordinated varietal trials (NCVT) throughout Pakistan and provincial coordinated cotton trials (PCCT) throughout the Punjab during 2012 to 2013 and 2013 to 2014.. This was on Ist in Punjab in Pakistan. CLCuV disease incidence (%),severity, intesivity, index and reaction of the genotypes was determined using the disease scale (*Table 1*) described by Akhtar *et al.,* 2010 and suggested by Farooq *et al.,* 2011.

CLCuV %age, disease incidence and disease index were calculated by using the following formula:

CLCuV disease incidence (%) = Sum of all disease ratings/total number of plants ×16.16.

Disease index= Disease %age x Disease severity/maximum severity value.

Table-1 Rating scale for cotton leaf curl virus (CLCuV) symptoms

0 = Complete absence of symptoms
1 = Small scattered vein thickening
2 = = Large groups of veins involved
3 = All veins involved
4 = All veins involved and severe curling

G.hirsutum was *G.stocksii* crossed in 2006 at Central Cotton Research Institute, Multan. Hybridization was done by manual emasculation and pollination under field conditions. Exogenous hormones were applied to control boll shedding. The crossed F1 seed was treated with colchicine 0.025%. Hexaploid to tetraploid plants were screened in field conditions with cytological studies and this concluded in Annual Summary Report of Institute that material is CLCuV resistant and can be utilized in our breeding programme to evolve virus resistant varieties.

RESULTS AND DISCUSSION

Increase in seed cotton yield is one of the major objectives in cotton breeding programmes. Genetic variability in cotton genotype with respect to seed cotton yield has been reported in various studies (Murtaza *et al.*, 1992a; Azhar and Rana, 1993; Sayal *et al*., 1996; Ahmad and Azhar, 1999).For assessing the yield potential and tolerance of genotypes against CLCuV, genetic material was screened in sick plot technique.

206 lines of [{2(G. *hirs*. x *G.anom*.) x *G.* ^{3}*hirs*. } x {2(*G. arbo*.x *G.anom*.)x *G.* ^{2}*hirs*. }] were screened through out cotton season under field conditions. Initially 82 families were showing resistance against CLCuV. These families were started to receive gradual susceptibility, one month after sowing and continued till 16 th September,2009 i.e 0-28 % in Table -1. 180 days after sowing 199 families were susceptible and seven were

resistance. At the end of the season re-sprouting of this material was checked and confirmed by Pathology Section.

Table-1 Screening of lines on the basis of sick plot technique during 2009-10:

Date	Days after sowing	Susceptible Families	Virus free families
19.05.2009	0	-	206
18.06.2009	30	124	82
*03.07.2009	45	164	42
18.07.2009	60	184	22
02.08.2009	75	188	18
17.08.2009	90	188	11
01.09.2009	105	197	9
16.09.2009	120	199	7
01.10.2009	135	199	7
16.10.2009	150	199	7
31.10.2009	165	199	7
15.11.2009	180	199	7

*CIM 496 was 100% virus infected during the month

Each plant of the resistant families was picked and ginned for the evaluation of lint %age and fibre characteristics. The range of lint %age and fibre characteristics of these elite plants are given in Table-2 Cyto-124 was 355-4/09 which comprises all desirable traits.

Table 2 Economic and fibre characteristics of Virus resistant lines during 2009-10

Family. No.	Range			
	Yield/ Plant (g)	Lint (%age)	Fibre length (mm)	Fibre fineness (µg/inch)
156/09	22.3-71.2	40.3-44.4	28.0-29.8	3.7-4.1
200/09	26.9-78.9	40.3-43.5	29.4-30.3	4.6-5.1
262/09	27.1-92.7	35.7-78.1	28.5-31.2	3.8-4.4
317/09	53.6-154.9	42.3-45.1	27.6-29.2	4.7-5.3
351/09	49.6-143.5	40.0-44.7	27.8-30.1	4.0-5.2
352/09	37.9-116.7	40.1-43.1	29.4-30.5	4.7-4.9
355/09 (Cyto-124)	**37.0-149**	**40.0-44.5**	**28.4-30.8**	**4.5-5.1**
CIM-496		**43.2**	**25.6**	**5.1**

On the basis of continuous performance, Cyto-124 was sent to NCVT during 2013-14 which is preliminary requirement for variety release.

All the NCVT strains showed highly susceptibility to cotton leaf curl disease as predicted in table-3. Minimum disease incidence, severity and disease index was recorded in Cyto-124. (Akhtar *et al*., 2002). The strain Cyto-124 and CIM-612 took more days to produce the symptoms, showing some tolerance against the disease and intensity of disease was also low. (Alim, 1997; Muhammad *et al*., 1998).

Table-3 Cotton Leaf Curl Disease Incidence, Severity and Disease Index in NCVT During 2013-14

NCVT Strain	Cotton Leaf Curl Disease		
	Incidence	Severity	Index
CIM-612	64.26	2.67	43.94
Cyto-124	**14.95**	**2.47**	**9.21**
DNH-105	100.00	2.96	73.91
CRIS-533	100.00	3.17	79.18
MPS-27	100.00	3.12	77.98
BH-177	100.00	3.07	76.64
TH-112/05	100.00	3.33	83.36
PB-896	100.00	3.04	75.88
Sun-2	100.00	2.99	74.86
CIM-573	100.00	3.04	76.06

Disease Index= Disease percentage x Disease severity/maximum severity value (4)

The material was also tested under greenhouse conditions. The material was grafted (petiole-graft technique) with cotton leaf curl virus infected leaves of cv. CIM-496. The observations were taken daily starting from one week after grafting. All the strains included in showed symptoms of CLCuD within 7 to 40 days after petiole-graft transmission. Data presented in tbale-4 showed that the strain Cyto-124 more days to produce the symptoms, showing some tolerance against the disease and intensity of disease was also low. The strains BH-177, TH-112/05 and CRIS-533 took few days to produce the symptoms and showed more susceptibility than others.

Table-4 Screening of NCVT Strains Against CLCuD Through Petiole-graft Transmission Technique during 2013-14.

Variety/ strain	No. of days taken to appear the symptoms (after grafting)	Intensity* 0-4*
CIM-612	30-38	2
Cyto-124	**32-40**	**1**
DNH-105	12-16	3
CRIS-533	10-12	4
MPS-27	12-15	3
BH-177	7-10	4
TH-112/05	7-10	4
PB-896	10-12	3
Sun-2	12-15	3
CIM-573	12-15	4

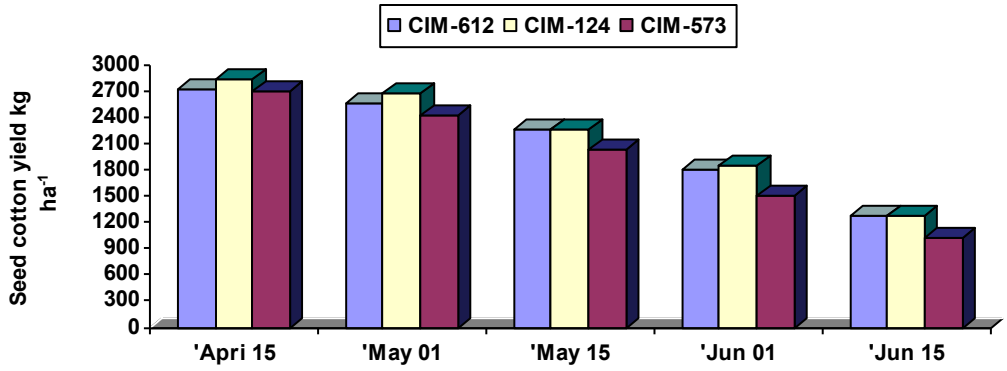
Two new genotypes i.e. CIM-612 and Cyto-124 with one standard CIM-573 were tested at five sowing dates starting from April 15th to June 15th at fifteen days interval. Experimental design was split plot. Sowing dates were kept in main plots and genotypes in sub plots with four repeats. Data on plant height, boll number, boll weight, seed cotton yield and CLCuD incidence (%age) recorded are given in Table 5.

Table 5 Effect of sowing dates on plant height, seed cotton yield, yield components and CLCuD incidence

Sowing dates	Genotypes	Plant height (cm)	Number of bolls plant^{-1}	Boll weight (g)	Seed cotton yield (kg ha^{-1})	CLCuD incidence %age 105 DAS
April 15	CIM-612	120	30	2.45	2724	1.39
	CIM-124	110	32	2.46	2848	1.58
	CIM-573	112	30	2.42	2710	63.85
May 01	CIM-612	116	28	2.49	2574	8.29
	CIM-124	108	30	2.50	2678	6.71
	CIM-573	102	28	2.41	2434	87.26
May 15	CIM-612	108	25	2.52	2278	28.59
	CIM-124	101	23	2.51	2266	25.91
	CIM-573	93	24	2.46	2034	99.38
June 01	CIM-612	99	20	2.55	1816	36.10
	CIM-124	96	21	2.56	1850	15.82
	CIM-573	89	19	2.49	1510	97.10
June 15	CIM-612	84	15	2.56	1284	100.00
	CIM-124	85	15	2.56	1280	19.50
	CIM-573	78	14	2.53	1026	100.00

DAS* = Days After Sowing

The data presented in Table 5 indicated that on over all average basis of sowing dates, CIM-124 produced significantly higher seed cotton yield than CIM-573. Averaged across the genotypes, plant height, boll weight and seed cotton yield decreased significantly as the sowing was delayed Among all sowing dates maximum boll weight was produced from 15th June sown crop (Fig.1).. Among genotypes, CIM-124 gave 12.5 higher seed cotton yield than CIM-573. These results were similar to Porter-PM et al., (1995), Shekara-BG et al., (1998), Srinivasan G. (2001) and Ali et al., (2004). They found that early sown cultivars produced gave more yield than late sown cultivars.



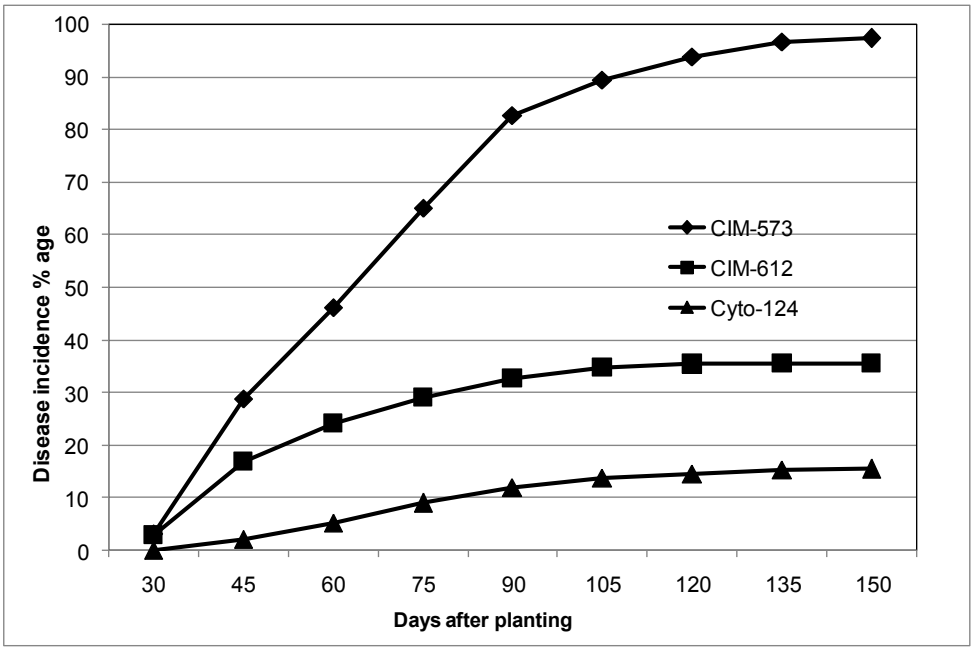


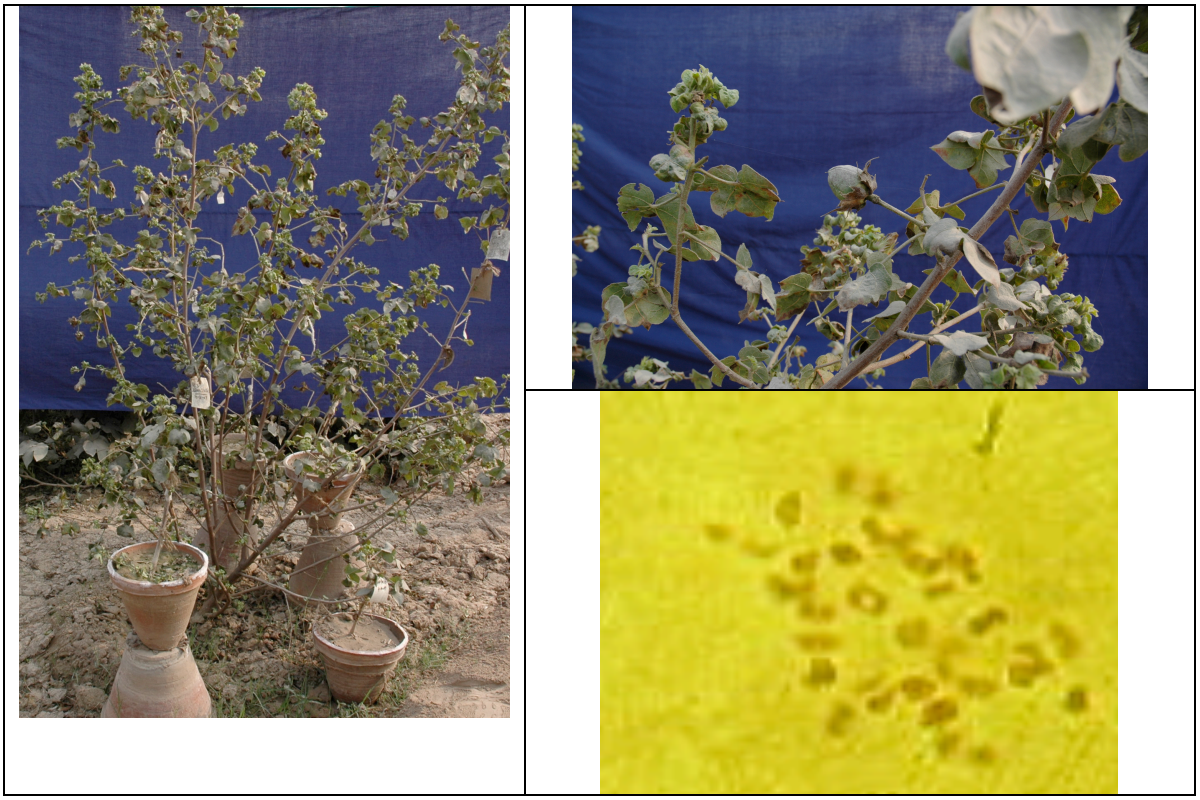
Fig-2 Effect of CLCuD Incidence as. influenced by planting dates and strain

A comparison among the varieties revealed that Cyto-124 was the least affected with CLCuD, on an average basis, in all sowing dates even when planted during the month of June. (**Fig-2**).

Fig-3 A photo graph of Cyto-124

G.stocksii was crossed with *G.hirsutm* in the fashion as described in Table-6. Tetraploid plants were also screened by grafting and field conditions, non of the plant showed symptoms of CLCuV compared with CIM-496 which was highly virus infected (Table-7) which is similar to our result presented in annual summary report 2006-07.

Steps	Crossing	Year of crossing	Characteristics
1	*G.hirsutum* x *G.Strocksii* **(52)** **(26)**	2006-07	-
2	**F_1** Triploid (39) (Sterile)	2007-08	**(strrile)**
3	2(*G.hirsutum* x *G.stocksii* **)** (colchcineiTreatment**)** **Hexaploid** (78) (Fertile)	2007-08	**(Partially Fertile)** Greenish Brown, softly hairiness, spares black glands, light yellow corolla colour,, faint petal spot, light yellow pollen colour, ovoid boll shape with 3-4 locks/boll.
4	2(*G.hir*. x *G.stockii*) x *G.hir.* **Pentaploid (**65) (Sterile)	2008-09	**(Partially Fertile)**
5	**Screened Tetraploid**	2009-10	**(Semi Fertlie)**



Hexaploid of 2(*G.hir*. x *G.stocksii*)

Morphology and chromosomal configuration of pentaploid

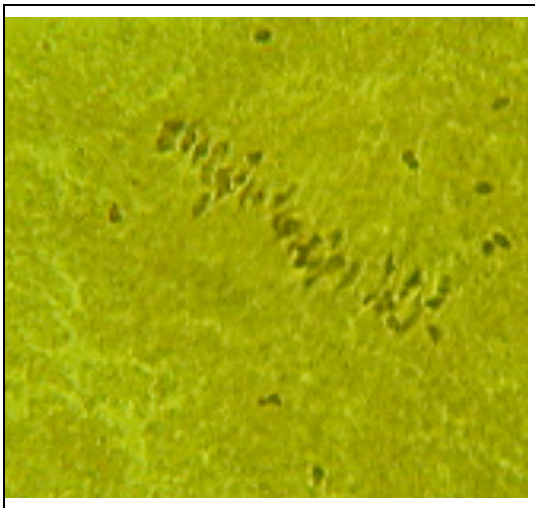
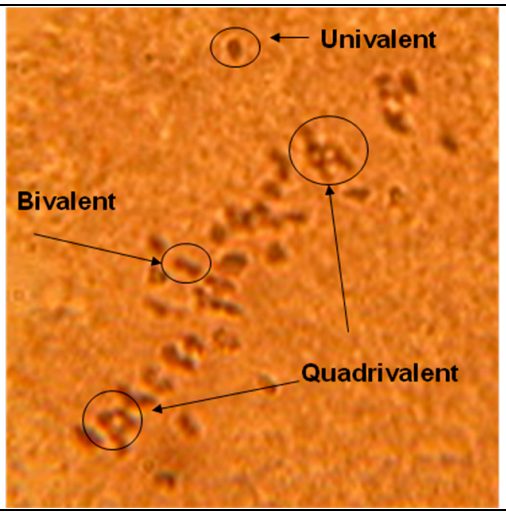
	
15 Is + 25 IIs =65	**7 Is + 23 IIs + 3 IVs =65**

Table-7 Screening of tetraploid plants against CLCuV.

Material	**No of plant tested**		**CLCuV response**
Tetrpolid (*G.hirsutumxG.sticksii*)	In field	Grafts	No virus was observed in field and grafts
	24	7	

The result indicated that tetraploid has more fibre strength(118.9), fibre fineness (4.8) than that of commercial cultivar CIM-554. Plants have many self bolls with a high number of seeds which are according to result of Basely (1942), Results are also in accordance to those obtained by Brown and Menzel (1952). This material having resistance to CLCuV is in our breeding programme to develop interspecific varieties having desirable traits with resistant to CLCuV.

Table-8- Comparative result of fibre properties of tetraploid and MNH-786

Material	Lint (%age)	Fibre Length (mm)	Uniformity Ratio	Strength (G/tex)	Fibre Fineness ($\mu g\ inch^{-1}$)
Tetraploid	**33.8**	**25.5**	**81.2**	**118.9**	**4.8**
CIM-554	**41.2**	**28.4**	**86.5**	**96.8**	**4.8**

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