TRANSFERENCE OF VIRUS RESISTANCE FROM *G. ARBOREUM* AND *G. ANOMALUM* INTO *G. HIRSUTUM*

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1 Introduction

Cotton, an important commercial crop, is extensively cultivated in Pakistan. It is infected by several pests and pathogens inducing different diseases. Among them cotton leaf curl virus is the most important, causing enormous losses to the crop (Khan and Ahmad, 2005). Leaf curl virus disease of cotton was first observed near Multan in the year 1967. This disease caused heavy damage to cotton crop during 90's. In 1991 the cotton area affected by this disease was about 35,000 hectares (Mehbub, *et al* 1992).In 1993-94 the cotton crop over 889000 hectares was damaged while there was record loss of yield of 1.88 million bales. This disease spread in Sindh in1997, in NWFP in1998 and in Balochistan in 2001.This disease is caused by a white fly transmitted begomovirus (cotton leaf curl virus) (Mansoor, *et al* 1993, Briddon and Markham, 2000).

This disease is characterized by either upward or downward curling of leaves. The veins of the leaves become thickened which are more pronounced on the underside. In extreme but not infrequent cases, formation of cup shaped leaf laminar outgrowth called enation appears on the underside of the leaf. Inter nodal distance is reduced and affected plants become stunted. There is reduction in the boll number and boll weight resulting in loss of yield. There is also a negative effect on fibre length, finess, strength and maturity etc. The white fly is the vector of this disease. There are about 118 alternate hosts of this disease. Amongst these are okra, sunkukra, gurhal/ chinese rose, til, potato, tomato, brinjal, pumpkin, cucumber, musk melon, water melon, *Luffa*, *Momordica*, sunflower, datura, gul-e-khera, *Convolvulus*(lehli), *Calotropis* (ak), *Amarantus* (karund), citrus, jute, *Geranium*(rattan jote), *Euphorbia*(hazar dani), *Phylanthus*(amla),*Ipomea*, *Capsicum*(red pepper) etc.. The Government and all research Institute/Stations through their all-out efforts tried to control this disease. The genetic stock available at CCRI Multan was carefully screened and it was found that three exotic cultivars viz., CP-15/2 ,LRA 5166 and CEDEX showed complete resistance to this disease. As a result of crossing between these three exotic cultivars and local varieties, virus resistant varieties viz., CIM-1100, CIM-448, CIM-443, CIM-446, CIM-482 and CIM-473 were developed and were approved by the Punjab Seed Council for general cultivation in the Punjab. These varieties covered about 80% area in the Punjab and yield loss decreased gradually from 2.1 to 0.082 million bales from 1996 to 2001 respectively. (Tariq *et. al.* 1992)

However due to new strain of virus commonly known as Burewala strain of cotton virus (BSCV) started damaging the cotton crop in district Vehari in general and in Burewala area in particular during 2001. All the cotton leaf curl resistant commercial varieties as well CP-15/2, LRA-5166 and CEDEX showed susceptibility to BSCV. 3338 genotypes of *Gossypium* germplasm from CCRI Multan, CRS Multan, CRI Faisalabad,. NIBGE Faisalabad, CCRI Sakrand and CRS Tandojam, planted at Cotton Research Station Vehari, were screened but none of these genotypes was found resistant to Burewala cotton leaf curl virus. The symptoms and alternate hosts of BSCV are similar to those of previous cotton leaf curl virus. After emergence of this new strain of virus the yield loss started increasing. The hot spots of this disease increased from 2.4% to 73.4% during the year 2001-2007. Similarly the area affected under this disease also increased from 4.45% to 70.26% during this period. At present no single variety is resistant to BSCV. This is alarming that the hot spots as well as area affected by this disease are

increasing every year and the situation is becoming verse. The cotton is the backbone of agricultural economy of Pakistan, which is being affected due to insurgence of BSCV and there is decease in production.

2 Review of Literature

The genetic improvement of crop requires the assimilation of germplasm resources, which can then be advanced into breeding materials. In order to accomplish this objective one approach is to make and utilize interspecific hybrids. The *Gossypium* species are the potential source of variability for cotton breeders. The wild species of *Gossypium* have numerous diverse and desirable characters including disease resistance, (especially cotton leaf curl virus, bacterial blight), insect pest resistance, drought and heat resistance/tolerance, fine and strong fibre.

In the past certain disease of cotton were successfully controlled through transferring desirable genes from *G. herbaceum*, *G. arboreum* and wild species into the *G. hirsutum*, *G. barbadense* etc.(Innes, 1992).Selected examples have been reviewed by Fryxell(1976a).

A disease of localized occurrence is cotton rust caused by *Puccinia cacabeta* Arth & Holw. (Blank and Leathers, 1963). It is of the importance in the South Western US and North Western Mexico. Fungicidal control of the disease is costly and not wholly satisfactory. L.M. Blank screened the available diploid species of *Gossypium* and found that *G. anomalum* and few cultivars of *G. arboreum* were rust resistant. Through inter-specific hybrids and artificial polyploids plus a back crossing scheme accompanied by certain screening for resistance he succeeded in transferring this resistant into *G. hirsutum*.

An other important disease in most cotton growing areas of the world is caused by *Xanthomonas malvacearum* (E.F. Smith) Dowson (Brinkenhoff, 1970) and is variously known as bacterial blight, angular leaf spot, and black arm disease. Knight (1950) in Sudan transferred a single bacterial blight resistant gene from *G. arboreum* to Sakel *G. barbadense*. This gene known as the B6, alone is weekly resistant, but in combination with each of several other B genes confers near immunity to the disease. Germplasm surveys have, over the years, discovered a whole series of monogenic sources of resistance , coming from *G. hirsutum*, *G. barbadense*, *G. herbecum*, *G. arboreum* and *G. anomalum.* Since the disease organism exists in a variety of strains at differing levels of virulence, plant breeders have generally combined two or more genes for resistance and, through selection, raised the effectiveness of the polygenic background in developing new blight .resistant genotypes. Satisfactory blight .resistant cultivars of both *G. hirsutum* and *G. barbadense* were available for many years.

The root-knot nematode (*Meloidogyne incognita acrita*) cuases serious cotton crop losses over a wide area. This organism is a parasite on cotton roots and causes a significant disruption of normal root function. In addition, the damage to the root system provides an entrance for other soil-born disease organisms, notably *Fusarium.* It is therefore appropriate to speak of damage resulting from the root-knot-nematode-*Fusarium*-wilt disease complex. This disease complex in the US cotton belt was recognized and described as early as 1892 and since that time cotton breeders have attempted to develop resistant varieties using a primitive race stock, of un-certain origin and resistant material was developed (Shepherd, 1974). Tolerance of root-rot was found by Muramoto (!969) in a hexaploid of *G.hirsutum* and *G.stutii.*

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.

Bird (1973) has defined resistance in cotton as the mechanism preventing colonization, effective growth and reproduction of a pathogen, entering the host plant. The term immunity is used, when no phenotypic symptoms occur, even after inoculation with virulent isolates of pathogens. The term escape is used to conclude the cases resulting reduced penetration, protection and alteration of environment creating un-favourable host pathogen interaction.

3 Materials and Methods

G. *arboureum* and *G. anomalum* were found resistant rather immune to cotton leaf curl virus. So *G. arboreum* and *G. anomalum* (diploids) were crossed as male with *G. hirsutum* (tetraploid) to transfer the virus resistance from both the diploid species into *G. hirsutum* background. Methods for developing virus resistant material are given below:-

i) *G. anomalum Wawra et. Payer*(2n= 26)-B1 was crossed with *G. hirsutum Linn*(2n=52)(AD)1as female. The resultant triploid hybrid was treated with 0.2% aqueous solution of colchicine for 72 hours using seedling dip method for doubling the chromosomes. The hexaploid was further crossed with *G. hirsutum* to make a pentaploid which was further back crossed four times to get a stable tetraploid.

ii) *G. anomalum Wawra et. Payer*(2n=26)-B1 was crossed with *G. arboreum*. *Linn*(2n=26)A2 The resultant diploid inter-specific hybrid was treated with 0.2% aqueous solution of colchicine for 72 hours using seedling dip method for doubling the chromosome. The resultant tetraploid hybrid was crossed and back crossed with *G. hirsutm* as detailed below.

G.arboreum

X

G.anomalum

2(G.arboreum x G.anomalum)

2(G.arboreum x G.anomalum)

x

G.hirsutum

2(G.arboreum x G.anomalum) x G.hirsutum

iii) Both the above mentioned species hybrids viz., [{.^{3}*hirs*. × 2 *(hirs*. ×*G. anom*.)} × {^{2}h*irs*. × 2 (*arbo*. × *anom*.)}] × *hirs*. were also crossed with each other.

The synthesized material was grafted with virus affected petioles of CIM-473 to check its virus resistance against BSCV in green house. Later on these resistant plants were shifted to field for assessment of their resistance against BSCV in field conditions. The number of the plants showing resistance in green house as well as in field was recorded.

Testing of species hybrids against virus through petiole grafting

4 Results

2431 plants of the material developed by species hybrids were grafted with virus affected of petiole of CIM-473 to check their virus resistance against BSCV in green house. Out of these 342 plants which did not show BSCV symptoms were transplanted in the field. Only 61 plants showed resistant against BSCV till maturity of crop. The result are given in Table 1.

Table 1 Screening of resistant material during 2006-07

Material	Total Number of plants grafted in green house	Number of Plants not Showing Symptoms and Transplanted in the field	BSCV Affected Plants In the Field	Resistant plant	%age resistance
^{4}*G. hirs.* × 2 (*hirs.* X×G. *anom.*).	303	13	11	2	0.66
^{4}*G. hirs.* X×(*G.arbo.* × G.*anom.*)	774	115	97 -	18	2.32
[{^{4}*G. hisr.* × 2 (*hirs.* × G. *anom.*)} × {^{2}*G. hirs.* × (*G.arbo.* × G.*anom*)}] ×*G. hirs*	1354	214	173 -	41	3.0
TOTAL	2431	342	281	61	

Table 1 shows that in the first *Gossypium* species hybrid viz., *G. hirs* × 2*(G. hirs* × *G. anom*), resistance to BSCV was 0.66%, where *G. anomalum* alone resistant to BSCV was used. While in the second combination viz., *G. hirs* × 2(*G. arbo* × *G. anom*), the resistance to BSCV was 2.32%. When two *Gossypium* i.e. *G. arboreum.* and *G. anomalum* resistant to BSCV were used. In the third combination i.e, [{^{3}*G. hisr.* × 2 (*hirs.* × G. *anom.*)} × {^{2}*G. hirs.* ×(*G.arbo.* × G.*anom*)}] ×*G. hirs.* where *G. anomalum* has been used twice and *G. arboreum* for once, the resistance against BSCV was 3.0%

The neutral Plants, neither grafted in greenhouse nor in the field were tested against BSCV. The results are given in table 2.

Table2 Screening of material in the field during 2006-07

Material	Total Number of plants In the field	BSCV Affected Plants In the Field	Resistant plant	%age resistance
^{4}*G. hirs.* × 2 (*hirs.* × G. *anom.*).	1231	1193	38	3.1
^{4}G. hirs. × (*G.arbo.* × G.*anom*)	121	85 -	36	29.7
[{^{4}*G. hisr.* × 2 (*hirs.* × G. *anom.*)} x {^{2}*G. hirs.* × (*G.arbo.* × G.*anom*)}] ×*G. hirs*	88	59 -	29	33.0
TOTAL	1440	1337	103	

Data given in the table 2 is also in agreement with that of table 1. The resistance to BSCV in the first, second hybrid, and in their combination was 3.1, 30.0 and 33.0% respectively.

Those plants which showed resistance against virus were picked, ginned and there fiber quality was evaluated. The economic and fibre characters of some of the resistant material are given in Table-3-6

Table 3 Performance of resistant plants of *G ^{4}hirs.* × 2 (*hir.* × *G. anom*

Hybrid No.	Seed cotton yield (g)	Lint (%age)	Fibre Length (mm)	Fibre fineness (µg/inch)	Fibre strength g/tex
G ^{4}hirs. × 2 (*hir.* x *G. anom.*)					
CP13/Z38	55.0	38.9	26.1	5.0	28.2
" Z40	204.3	40.2	29.8	4.5	27.3
G ^{3}hirs. x 2 (*hi.r* × *G. anom.*)					
CP13 (2004)	287.4	37.4	26.9	4.6	23.7
G ^{2}hirs. × 2 (*hir.* × *G. anom.*)					
P6 (2003)	37.6	36.9	26.2	4.4	27.1

The material, synthesized above, showed progressive increase in yield and ginning out turn percentage with improved fibre qualities.

Table 4 Performance of resistant plants of *G. ^{4}hirs.* × *2* (*G. arbo.* × *G. anom.*)

Plant No.	Seed cotton yield (g)	Lint (%age)	Fibre length (mm)	Fibre fine-ness (µg/inch)	Fibre strength (g/tex)
Resistant plants of *G. ^{4}hirs.* × *2* (*G. arbo.* × *G. anom.*)					
CP-37/Z50	117.4	42.2	30.4	3.3	36.1
" Z51	47.4	41.9	29.8	3.2	33.7
" Z52	117.8	42.2	31.0	3.9	34.5
" Z52A	25.06	44.0	30.5	4.0	31.1
" Z54	122.2	42.0	28.2	5.3	27.8
CP-38/Z55	47.6	45.9	28.3	2.9	36.4
" Z56	87.0	36.3	29.9	4.3	30.1
AB1/Z61	57.2	39.9	29.7	3.3	31.8
" Z67	41.0	34.1	33.3	3.5	34.6
" Z69	218.4	39.5	31.3	3.4	35.5
Neutral plants of *G. ^{4}hirs.* × 2 (*G. arbo.* × *G. anom.*)					
CP-29	184.3	43.2	28.6	5.0	26.0
"	96.0	41.5	28.4	4.4	31.2
"	225.5	43.9	28.2	5.8	24.9
CP-30	166.6	34.6	31.8	3.5	35.3
"	112.2	38.3	30.7	4.2	34.6
"	30.4	39.5	31.5	4.3	32.8
CP-31	75.3	43.6	28.1	3.8	28.7
"	33.0	46.6	28.8	4.1	29.6
"	58.4	41.6	30.2	3.6	32.2
CP-32	292.0	38.8	31.6	3.3	33.0
CP-36	108.2	45.4	29.0	3.0	37.8
CP-37	230.0	45.0	29.	3.8	31.6
"	71.0	43.7	31.0	3.2	32.8
CP-38	62.0	46.3	26.5	4.8	25.6
AB1	74.7	41.8	28.8	3.1	32.6
"	74.2	39.6	27.8	3.4	30.9
"	64.3	40.0	29.4	3.4	30.8
P-22 (2004)	24.0	33.3	31.1	3.2	32.8
CIM-496 (C.S.)	90.3	41.2	28.9	4.8	26.5
CIM-506 (C.S.)	86.1	37.2	28.5	4.7	26.3

The material mentioned in table -4 showed good ginning out-turn percentage, long , fine and strong fibre.

Table 5 Performance of of resistant plants of [{*G.* ^{3}*hirs.* × 2 (*G. hir.* x*G. anom.*)} × {*G.* ^{2}*hirs.* X 2(*arbo.* × *G. anom.*)}] × *G. hir.*

Plant No.	Seed cotton yield/plant (g)	Lint (%age)	Fibre length (mm)	Fibre fineness (µg/inch)	Fibre strength (g/tex)
CP-3/Z2	23	44.1	28.9	3.6	32.2
CP-4/Z3	254	38.4	29.4	3.9	29.1
" Z4	114	37.7	33.4	3.5	32.5
" Z5	418	41.9	32.3	4.1	29.1
" Z6	303	39.0	34.3	3.4	31.9
" Z8	116.6	36.7	32.0	3.1	32.5
CP-5/Z15	60.3	35.2	33.8	3.6	29.9
" Z16	76.3	35.9	31.8	3.5	29.9
" Z18	141.0	35.1	30.0	4.1	29.1
CP-7/Z24	86.0	38.6	30.4	4.5	33.9
" Z27	148.2	35.4	30.9	4.4	30.2
" Z28	153.4	45.6	28.3	3.8	30.3
" Z29	26.4	41.7	27.8	3.9	31.6
CP-8	188.8	39.4	29.0	4.8	27.0
CP-11/Z32	120.4	45.3	31.1	3.4	36.0
" Z34	144.3	37.6	32.3	3.6	34.2
" Z42A	89.0	40.1	30.0	3.3	34.5
CP-12/Z36	234.6	35.4	30.0	4.5	28.4
" Z37	153.0	39.2	30.3	3.3	33.4
CP-17/H3	14902	38.6	29.1	4.3	28.7
" H7	83.8	37.8	29.3	4.1	28.7
" H12	200.3	44.4	28.1	3.7	29.2
" H13	205.0	36.0	29.4	4.4	30.8
" H14	353.5	39.5	30.9	4.0	29.8
" H15	64.2	41.1	28.5	3.7	29.5
" H16	130.0	35.4	30.8	4.0	30.0
CP-17	293.5	39.8	30.7	3.7	30.3
CP-24/Z47	261.8	35.0	31.7	3.2	32.2
P6 (2003)	37.6	36.9	26.2	4.4	27.1
P-22 (2004)	24.0	33.3	31.1	3.2	32.8
CIM-496 (C.S)	90.3	40.2	28.1	4.8	26.5
CIM506 (C.S.)	86.1	37.2	27.8	4.9	26.3

Data in the table -5 shows that good combinations of economic and fibre characteristics are available in this material synthesized through multiple species hybrids.

Table 6 Performance of of resistant plants (Neutral plants)

Plant No.	Seed cotton yield/plant (g)	Lint (%age)	Fibre length (mm)	Fibre fineness (µg/inch)	Fibre strength (g/tex)
CP-5	61.5	35.8	31.7	3.1	37.6
CP-6	166.3	37.3	29.4	3.9	34.6
CP-11	129.0	38.8	30.4	3.5	34.7
CP-17	336.5	35.5	30.6	4.1	29.9
"	80.6	39.7	30.3	3.9	31.4
"	141.1	38.6	30.1	4.6	30.0
"	131.0	38.9	29.9	4.1	31.0
"	97.8	40.2	29.4	4.2	28.9
"	207.8	37.5	30.7	4.1	31.4
CP-21	59.5	44.9	32.2	2.8	32.6
CP-24	100.8	43.3	29.5	3.2	32.7
"	103.0	40.8	30.9	3.6	35.0
CP-25	121.3	50.5	27.5	4.1	29.9

Data in table-6 revealed that outstanding combinations of different economic and fibre characteristics are present in this material,

The cotton leaf curl virus resistant plants were selfed and seeds of this material were sown in the field in plant to progeny row to find out homozygous plants for virus resistance. The work is in progress.

Interspecific hybrids with good fruiting & opening

5 Discussion

Knight(1950) transferred bacterial blight resistance combing several B genes in Sakel (*G. barbadense*) as the disease organism existed in a variety of strains at differing levels of virulence. Our results are also in agreement with those of Knight It is revealed from the results given in table-1 and- 2 that resistance to BSCV may be polygenic in nature

This material is in and F3 level. At present heterozygous BSCV resistant plants are obtained which segregate in the next generation. This may be due to more than two species involved in this material. As the polyploidy level of the synthesized hybrid was changed through colchicines treatment, then crossed and back crossed with the recurrent parent i.e. *G. hirs*, there is some disturbance amongst these hybrid. Next year when this material is sown in the field, it is expected that homozygosity in this material against BSCV will be obtained.

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