GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Introgression of genes for cotton leaf curl virus resistance and increased fiber strength from *Gossypium stocksii* into upland cotton (*G. hirsutum*)

W. Nazeer[1,2], S. Ahmad[2], K. Mahmood[2], A.L. Tipu[2], A. Mahmood[3], B. Zhou[1]

[1]National Key Laboratory of Crop Genetics and Germpalsm Enhancement, Cotton Research Institute, Nanjing Agricultural University, Nanjing, China
[2]Cotton Research Station, Multan, Pakistan,
[3]Cotton Research Institute, Faisalabad Pakistan

Corresponding author: B. Zhou
E-mail: baoliangzhou@njau.edu.cn

Genet. Mol. Res. 13 (1): 1133-1143 (2014)
Received March 30, 2013
Accepted August 20, 2013
Published February 21, 2014
DOI http://dx.doi.org/10.4238/2014.February.21.2

ABSTRACT. Cotton leaf curl virus disease is a major hurdle for successful cotton production in Pakistan. There has been considerable economic loss due to this disease during the last decade. It would be desirable to have cotton varieties resistant to this disease. We explored the possibility of transferring virus resistant genes from the wild species *Gossypium stocksii* into MNH-786, a cultivar of *G. hirsutum*. Hybridization was done under field condition at the Cotton Research Station, Multan, during 2010-11. Boll shedding was controlled by application of exogenous hormones. F_1 seeds were treated with 0.03% colchicine solution for 6 h and germinated. Cytological observations at peak squaring/flowering stage showed that these plants were hexaploid, having 2n = 6x = 78 chromosomes. The F_1 plants showed intermediate expression for leaf size, leaf area, petiole length, bracteole number

and size, bracteole area, bracteole dentation, flower size, pedicel size, and petal number and size. Moreover it possessed high fiber strength of 54.4 g/tex, which is 54% greater than that of the check variety, i.e. MNH-786 (*G. hirsutum*). The F_1 population did not show any symptom of CLCuVD in the field, tested by grafting with CLCuVD susceptible rootstock (var. S12). We conclude that it is possible to transfer CLCuVD resistance and high fiber strength from *G. stocksii* to *G. hirsutum*.

Key words: Gene introgression; Cytogenetics; *Gossypium stocksii*; Cotton leaf curl virus resistance; *Gossypium hirsutum*

INTRODUCTION

Cotton can be infected by various insects, pests and pathogens, causing different diseases. Among them, cotton leaf curl virus (CLCuV) is the most damaging disease, causing enormous losses (Khan and Ahmad, 2005). With the passage of time, CLCuV has spread to all provinces of Pakistan (Tariq, 2005). It has caused a decrease of 9.45 million cotton bales during the last decade. Reduction in yield of tolerant and susceptible varieties is reported to be 50 and 85-90%, respectively (Hussain, 1995; Khan et al., 2001). Moreover, higher fiber strength is very important for the textile sector. The available commercial varieties in Pakistan do not have high fiber strength (≤32 g/tex).

Numerous scientists have worked on interspecific hybridization for transferring resistant genes for favorable traits from wild diploid species into tetraploid cultivated cotton (Ahmed et al., 2011). Blank and Leathers (1963) transferred resistant genes against cotton rust, caused by *Puccinia cacabat*, from *Gossypium anomalum* 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 researchers (Bao-Liang et al., 2003; Ahmad et al., 2011) have been successful in interspecific introgression of *Gossypium hirsutum* L. and *Gossypium arboreum* L. Similarly, Knight (1957) and Brinkerhoff (1970) introgressed resistant genes B_6 found in the 'A' genome of *Gossypium arboreum* L. against bacterial blight caused by *Xanthomonas malvacearum* into *Gossypium barbadense* L. Breeders have also achieved developing the most resistant commercial variety 'Auburn 56' to root knot nematode (*Meloidogyne incognita*) in U.S. cotton through transgressive segregation (Shepherd, 1974). Sacks and Robinson (2009) introgressed resistance to *Rotylenchulus reniformis* into the tetraploid 2 $(AD)_1$ through crossing a resistant diploid A_2-genome *Gossypium arboreum* L. accession (A_2-190) with a hexaploid 2 [$(AD)_1$ D_4] bridging line (G 371) to obtain a tetraploid triple species hybrid.

Culp and Harrel (1973), Green and Culp (1990), and Culp and Green (1992) focused on transferring genes for extra fiber strength starting in 1946, which was based on Beaseley (1940) triple hybrid [(*G. thurberi* x *G. arboreum*) x *G. hirsutum*] to improve upland cotton. *Gossypium* germplasm with extra fiber strength was successfully developed by them. They pointed out that the negative association between lint yield and fiber strength was eliminated and fiber strength was increased.

At present not a single variety of *G. hirsutum* shows resistance to the Burawala strain of CLCuV (Ahmad et al., 2010), but *G. stocksii* is resistant to cotton leaf curl virus disease and possesses excellent fiber strength. Ansari (1958) reported that the plants of *G. stocksii* were free of pests and diseases and speculated on the possibility of the usefulness of this species in producing disease-resistant cultivated cotton by hybridization. Ahmad et al. (2011) found *G. stocksii* resistant to cotton leaf curl virus disease. This species is found in the desert area of Sindh, Pakistan, Arabia, and East Africa. It is a small decumbent shrub, where the stem is softly tomentose with profusely black glands. Seeds are densely covered with firmly attached fine, brownish and short fibers (Knight, 1949; Saunders, 1959, 1961), cytologically displaying the E_1 genome (2n = 2x = 26) (Douwes, 1953; Phillips, 1966). Arutyunova and Volkova (1987) reported that the F_1 hybrid between *G. hirsutum* and *G. stocksii* had low boll setting on selfing, but that on crossing with *G. hirsutum*, there was high boll setting. Liang et al. (2002) developed hybrids BC_2 F_7 generation from the hexaploid of *G. hirsutum* x *G. stocksii*. This material had high fiber strength. In view of the importance of CLCuV in Pakistan and the role of fiber strength in the textile industry, studies were conducted to explore the possibility of transferring CLCuV resistance and fiber strength genes from *G. stocksii* (2n = 2x = 26) into cultivated upland cotton (*Gossypium hirsutum* L. 2n = 4x = 52). The objective was to develop a cotton hybrid that can resist cotton leaf curl virus disease and has high fiber strength.

MATERIAL AND METHODS

Commercial cotton cultivar var. MNH-786 (*G. hirsutum*) (Figure 1A) was crossed with *G. stocksii* (Figure 1B) in September, 2010 at the Cotton Research Station Multan. Hybridization was done by manual emasculation and pollination under field conditions. Emasculation was carried out in the evening and emasculated flowers were pollinated on the following morning. Exogenous hormones, including 50 mg/L gibberellic acid and 100 mg/L naphthalene acetic acid, were applied at the base of pedicles after 24 h of pollination for three consecutive days to control boll shedding.

Colchicine treatment

The number of bolls set was counted at the time of harvest. The crossed (F_1) seeds obtained were then treated with 0.03% colchicine for 6 h and germinated in the germinator at 32 ± 1°C in March, 2011.

Morphological characteristics

Plant habit, stem color, leaf texture, shape and hairiness, bracteole size, corolla color, petal spot, position of staminal column, anther color, and dehiscence of parents and F_1 were recorded.

Pollen viability estimation

To analyze the pollen grains of F_1 hybrids for viability, flowers were collected in the morning. A 2% solution of 2,3,5-triphenyl tetrazolium chloride in 60% sucrose was prepared. A drop of the solution was placed on a glass slide and pollen from freshly picked flowers were

agitated with a needle for 30 s to get a uniform immersion in the stain. Excessive pollen on the slide was wiped away and slides stored at room temperature. Pollen counts were performed after 24 h of staining. Stained pollen grains were considered to be viable.

Cytological studies

Young flower buds of F_1 hybrids were collected and fixed in Carnoy's solution at 8:00 to 9:00 am. After 24 h, these buds were preserved in 70% ethanol. Four anthers were squashed on a slide with a drop of 2.5% acetocarmine and examined under the microscope. The chromosome behavior of F_1 was critically examined under the microscope and photographs were taken using a camera mounted on a Labomed microscope.

Screening of F_1 hybrid plants against cotton leaf curl virus

Resistance in F_1 hybrid plants to cotton leaf curl virus was assessed on the basis of field observation, in the presence of a high population of whitefly, a carrier of CLCuV, through grafting and field conditions. In grafting, the scion was made of branches taken from the F_1 hybrid while stock was from virus susceptible *Gossypium hirsutum* L. var. S-12.

Measurement of fiber qualities

Seed cotton was harvested from F_1 hexaploid plants. Fiber was ginned by a roller ginning machine and fiber quality was measured on an USTER HVI Spectrum-1 instrument.

RESULTS

Crossing pattern

The crossing pattern of *G. hirsutum* with *G. stocksii* is given in Table 1. To have sufficient quantity of inter-specific hybrid seed for cytological studies as well as field cultivations, the maximum possible pollinations were performed, and as a result of 438 pollinations, only 15 bolls were found to be successful yielding 28 seeds. The low number of boll formation and seed setting were clearly attributed to inter-specific crossing. Thus, the boll setting percentage for *G. hirsutum* x *G. stocksii* was 3.4%. The impact of wide crossing was extended even after boll formation in the form of low germination percentage. The seeds obtained, treated with 0.03% colchicine, were germinated to raise seedlings, and 12 seedlings were successfully obtained. Out of 12 seedlings, only 4 hexaploid ($2n = 6x = 78$) plants (Figure 1C) reached maturity, which represented 42.9% germination and a 33.3% success rate of hexaploid development.

Pollen viability of hexaploid [2(*G. hirsutum* x *G. stocksii*)]

The pollen viability of *G. hirsutum* and *G. stocksii* was 87.6 and 82.3%, respectively, whereas F_1 hexaploid plants showed 51.1% pollen viability, which was 36.5% lower than for *G. hirsutum* and 31.2% lower than for *G. stocksii* (Table 2). Pollen viability of the F_1 hexaploid is shown in Figure 2.

Table 1. Detail of pollination and boll set for development of F_1 hybrid.

Parentage	No. of pollinations attempted	No. of bolls set	% age set	No. of seeds obtained	No. of seeds germinated	Germination % age	Success rate of Hexaploid development
G. hirsutum x *G. stocksii*	438	15	3.4	28	12	42.9	33.3 %

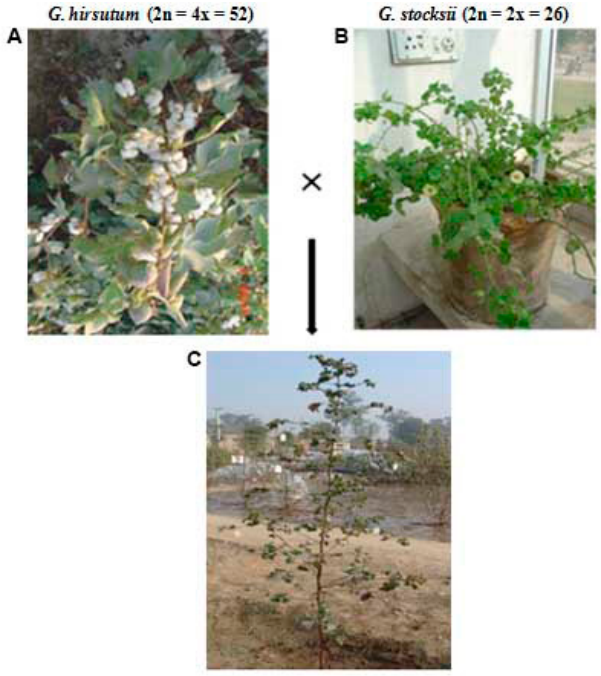

Figure 1. Hybridization between *G. hirsutum* x *G. stocksii* **A.** Female parent **B.** Male parent **C.** F_1 *G. hirsutum* x *G. stocksi* (2n = 6x = 78).

Table 2. Pollen viability of F_1 Hybrid 2 (*G. hirsutum* x *G. stocksii*) and its parents.

Trait	*G. hirsutum* L.	*G. stocksii* L	F_1 hybrid
Pollen viability (%)	87.6	82.3	51.1

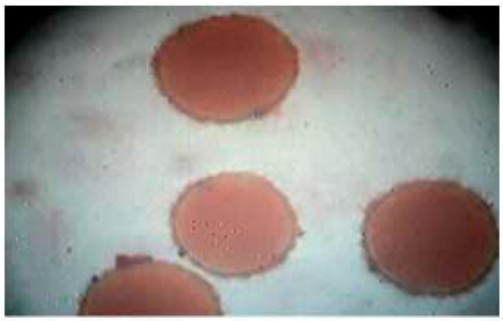

Figure 2. Pollens of hexaploid (51.1% viability).

Morphological characteristics of hexaploid [2(*G. hirsutum* x *G. stocksii*)]

The F_1 hybrid plants of the mentioned cross combination were intermediate between the two parents for most of the morphological characteristics, namely leaf characteristics (leaf size, leaf area, leaf lobation, and petiole length); flower characteristics (flower size, pedicel length, petal number and size, petal spot, position of staminal column, and pollen color); boll characteristics (bracteole size, bracteole area, bracteole teeth, boll size, boll shape, and number of locks/bolls). Maternal effects were observed for black glands, leaf color, leaf lobation, leaf hariness, and pistil size, whereas stem hairiness and corolla color were under the paternal effects (Table 3).

Table 3. Morphological characteristics of F_1 Hybrid 2 (*G. hirsutum* x *G. stocksii*) and its parents.

	Morphological characteristics	*G. hirsutum* L.	*G. stocksii* L.	F_1 hybrid
Stem characteristics	Stem color	Green	Greenish	Greenish brown
	Stem hairiness	Hairy	Softly tomentose	Softly tomentose
	Black glands	Sparse	Profuse	Sparse
Leaf characteristics	Leaf color	Green	Light green	Green
	Leaf size	Large	Small	Medium
	(length x breadth)	(10.9 cm x 10.1 cm)	(3.6 cm x 4.2 cm)	(5.8 cm x 6.1 cm)
	Leaf area	73.6 cm	10.1 cm	23.4 cm
	Leaf lobation	3-7 shallow lobed	3-5, shallow lobed.	3-7, deep
	Leaf texture	Herbaceous	Herbaceous	Thick
	Leaf hairiness	Hairy	Pubescent	Hairy
	Petiole length	Long (11.2 cm)	Short (3.1 cm)	Medium (5.7 cm)
Flower characteristics	Flower size	Large	Small	Medium
	Pedicel length	Long (1.2 cm)	Short (0.3 cm)	Medium (0.5 cm)
	Calyx	5 sepals farming a cup with teeth	5 sepals light green forming a cups with teeth	5 sepals forming a cups with teeth
	Corolla colour	Creamy	Light yellow	Light yellow
	Petal number and size	5, large (4.2 cm x 3.8 cm)	5, small (2.4 cm x 1.5 cm)	5, medium (4.0 cm x 3.5 cm)
	Petal spot	Absent	Dark prominent	Faint
	Position of staminal column	Long (1.8 cm)	Short (0.9 cm)	Medium (1.5 cm)
	Anther dehiscence	Normal	Normal	Normal
	Pollen colour	Creamy	Yellow	Light yellow
	Pistil size	Long (3.0 cm)	Medium (1.3 cm)	Long (2.8 cm)
Boll characteristics	Bracteole number	3	3	3
	Bracteole size	Large (3.3 cm x 2.3 cm)	Small (1.2 cm x 1.9 cm), united at base	Medium (2.6 cm x 2.3 cm)
	Bracteole area	4.3 cm	1.24 cm	3.2 cm
	Bracteole dentation	7-13, shallow	9-13 shallow	8-11 shallow
	Boll size	Large	Small	Medium
	Boll shape	Oblong	Round	Ovoid
	Boll surface	Glazes with few small glands	Course	Course with distinct glands
	Lock/boll	4-5	4-5	3-4
	Seeds/locule	7-9	2	1-4
	Seeds /boll	26.3	5.4	6.8

Leaves were thick, pubescent and intermediate in size, shape, lobation, and petiole length (Figure 3A). Flowers, bracteoles (size, shape, and teeth) and petals (size and color) of F_1 were also intermediate between the two parents, i.e., *G. hirsutum* and *G. stocksii* (Figure 3B, C, D). Anther dehiscence was normal and pollen color light yellow (Figure 3E).

Bolls were ovoid, coarse on surface and distinctly gland dotted (Figure 3F). Seeds had densely green fuzz. Fibers were of higher strength, light brownish, and firmly attached on the surface of the seed.

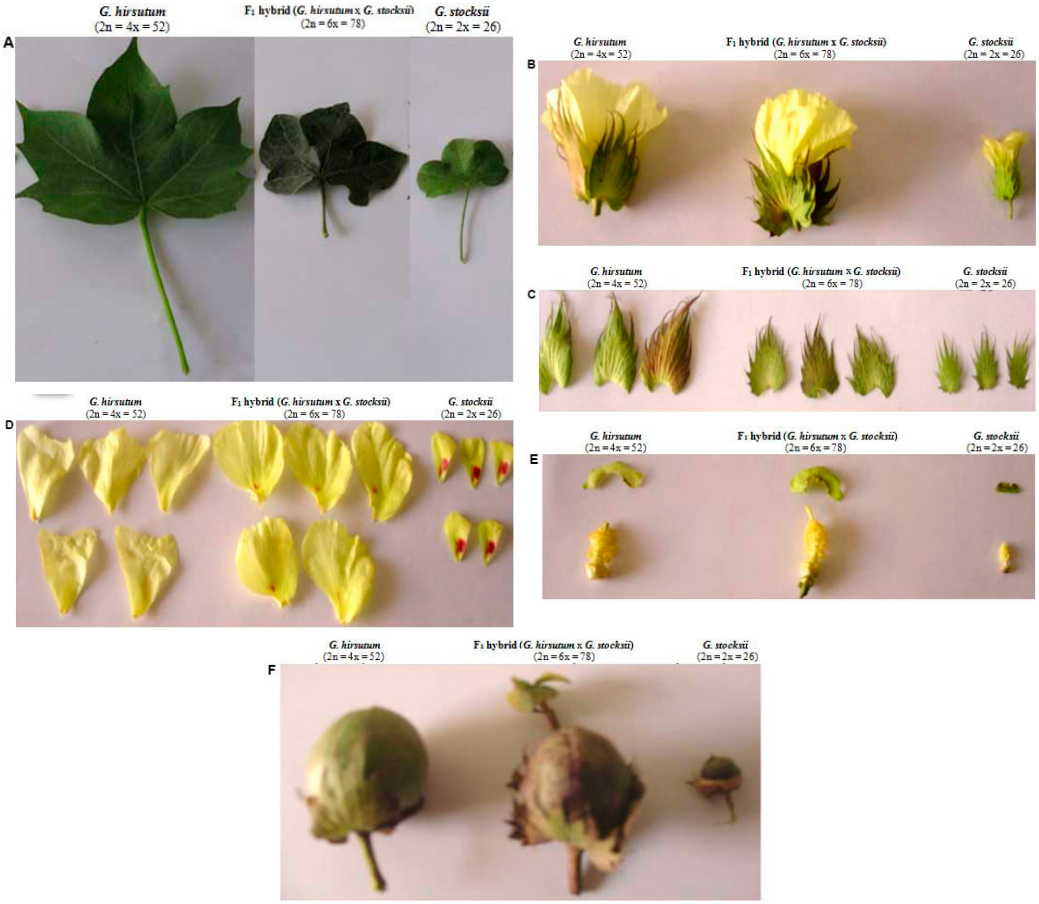

Figure 3. Morphological characteristics of F_1 hybrid (*G. hirsutum* x *G. stocksii*) and its parents **A.** Leaves; **B.** Flowers; **C.** Bracteoles; **D.** Petals; **E.** Calyx and Reproductive organ; **F.** Boll.

Cytological studies

Microscopic examination of pollen mother cells (PMCs) at metaphase-1 revealed that the hybrid plants had 78 chromosomes and genome $2[(AD)_1 E_1]$ with an average chromosomal configuration of 0.8 I + 38.3 II + 0.02 III + 0.09 IV per cell at M-1, in which 70.3% possessed 39 bivalents (Table 4). The frequency of chromosome pairing in the hexaploid was the highest, and gamete formation could be expected to be regular, while the frequency of univalents and multivalents was the lowest (Figure 4).

Development of microspores was normal. The pollen grains seemed to be normal but their pollen viability was 51.1%.

Distribution of PMCs at the sporad stage (Figure 5) showed that 89.8% PMCs contained 4 microspores (Table 5). Although the frequency of tetrad formation was the highest, pollen viability was 51.1%, leading to low boll setting on selfing in the hexaploid.

Table 4. Frequency of chromosomes at metaphase-1 of the hexaploid.

PMCs No.	I	II	III	IV	%
90	0	39	0	0	70.3
9	2	38	0	0	7.0
3	1	37	1	0	2.3
12	2	36	0	1	9.5
14	4	37	0	0	10.9
Range	0-4	36-39	0-1	0-1	
Average of 128 cells	0.8	38.3	0.02	0.09	

I Monovalents, II Bivalents, III Trivalents, IV Quadrivalents.

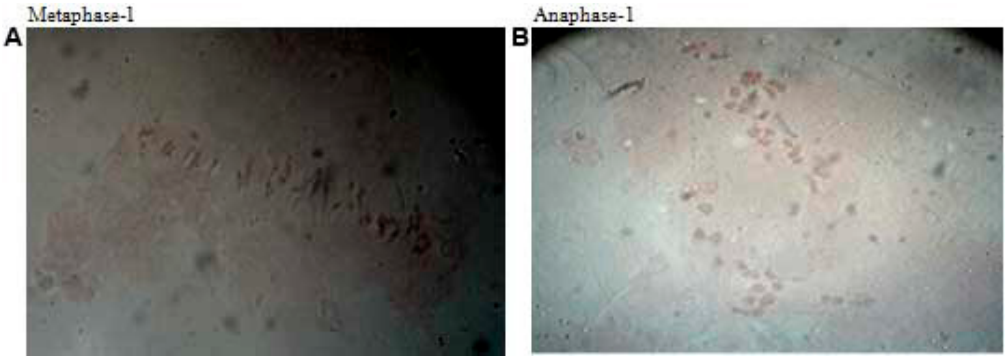

Figure 4. Meiosis in F_1 hybrid (2 I's +36 II's +1IV's) **A.** Metaphase-1 **B.** Anaphase-1.

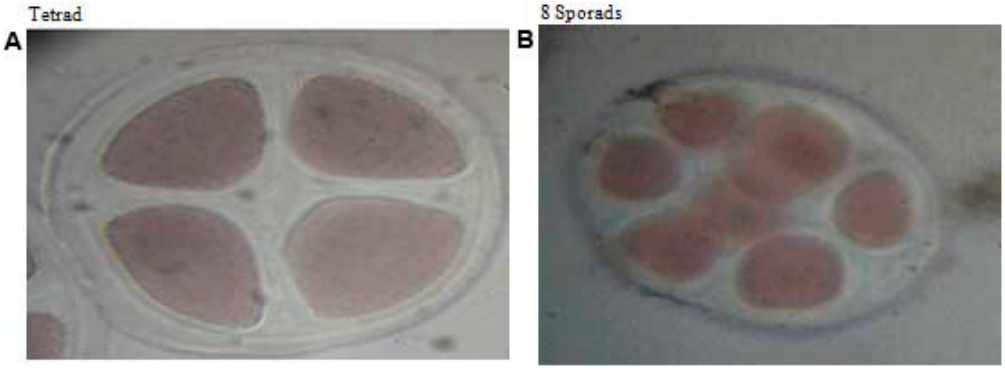

Figure 5. Sporad formation **A.** A Pollen mother cell with Tetrad **B.** A Pollen mother cell with 8 Sporads.

Table 5. Distribution of pollen mother cell at Sporad Stage.

Sporad	Percentage
I	2.3
II	1.0
III	1.0
IV	89.8
V	3.3
VI	1.3
VII	0.3
VIII	0.6
IX	0.3

Screening of F_1 hexaploid hybrid against cotton leaf curl virus disease (CLCuV)

F_1 hybrid plants of *G. hirsutum* x *G. stocksii* were screened by grafting and under natural field conditions. In field conditions, none of the 4 hexaploid plants showed symptoms of CLCuV compared with variety S-12, which was highly virus infected. All 17 grafts were also asymptomatic to CLCuV (Table 6).

Table 6. Screening of F_1 hybrid plants against CLCuV.

Parentage	No. of plants tested		CLCuV response
	In field	Grafts	
2 (*G. hirsutum* x *G. stocksii*) 2[$(AD)_1$, E_1]	4	17	No virus was observed in field and grafting

Fiber properties of hexaploid

The results of fiber traits, i.e., lint percentage, staple length, uniformity ratio, fiber strength, elongation percentage, and micronaire, are listed in Table 7, which indicated that F_1 hexaploid possessed greater fiber strength (54.4 g/tex), fiber elongation (6.7%) and fiber fineness (5.6 µg/inch) than that of commercial cultivar MNH-886.

Table 7. Comparative results of fiber properties of Hexaploid and MNH-786 (*Gossypium hirsutum*).

Material	Lint %	Staple length (mm)	Uniformity ratio	Strength (g/tex)	Elongation (%)	Micronaire (µg/inch)
Hexaploid	33.1	25.9	81.0	54.4	6.7	5.6
MNH-786	39.0	27.8	85.6	35.4	5.1	5.8

DISCUSSION

The crossability studies showed that there was only 3.4% boll setting (Table 1). Malik and Sheikh (1974a,b) reported that there was 2.3 and 2.8% boll setting in the crosses *G. arboreum* x *G. stocksii* and *G. anomalum* x *G. stocksii*, respectively. However, when *G. stocksii* was used as the female and was crossed with *G. arboreum,* boll setting was 15.0%. Pollen grains of *G. stocksii* showed some sort of incompatibility when *G. arboreum, G. hirsutum*, and *G. anomalum* were used as the female. This means most pollen tubes of *G. stocksii* could not traverse the stigma and style of female parents and cause fertilization.

In general, the hexaploid hybrid plants were more vigorous and were intermediate for several traits between the two parents. Chromosomal configuration of the hexaploid showed 0.8 I + 38.3 II + 0.02 III + 0.09 IV per cell; 70.3 % of PMCs had 39 II and 11.8 % PMCs had multivalent association at M-I (Table 5), indicating that heterogenetic recombination of chromosomes occurred between $(AD)_1$ and E_1 genomes. We interpret this as evidence of *G. stocksii* (E_1) having a distant relationship with *G. hirsutum* $(AD)_1$. This hexaploid of *G. hirsutum* x *G. stocksii* was capable of self fruit. As a consequence of high frequency of pairing in the hexaploid, gamete formation could be expected to be rather regular. Therefore, the proportion of normal PMCs containing 4 microspores was as high as 89.8% (Table 5). Our cytological results are in agreement with those of Brown and Menzel (1952), who reported that 2[$(AD)_1$ E_1] hexaploids were prolific, readily setting many self bolls with a high number of seeds. Beaseley (1942) also

reported that hexaploids had nearly perfect pairing and high fertility. As regards fertility, our results are in contrast to those obtained by Brown and Menzel (1952) and Beasely (1942). In our case, the hexaploid showed shy bearing, which might have been due to low pollen viability (51.1%) (Table 2), but our results on fertility are in agreement with those of Arutyunova and Volkova (1987) who reported that F_1 hybrids had low boll setting. Umbeck and Stewart (1985) reported hybrids of *G. stocksii* with *G. hirsutum* and *G. arboreum* and found little homology to either the A or D subgenome of *G. hirsutum* or the A genome of *G. arboreum*. Hybrids involving *G. stocksii* showed a little homology to either the A or D subgenome of *G. hirsutum* or the A genome of *G. arboreum*. Due to relative similarity of chromosome size between E_1 and the A chromosome, the type of syndetic pairing could not be ascertained. In the study of Katterman and Ergle (1970), the C genome species had a very large chromosome, the B, E, and F genome species had large chromosomes that were slightly larger than those of the A genome, and the A genome species had moderately large and the D genome species small chromosomes. Poisson et al. (1969) produced a hybrid of *G. anomalum* x *G. stocksii* and reported evidence of chromosomal homology between these two species by crossing this hybrid with *G. hirsutum*. They produced a series of lines (n = 27) obtained by the addition of one chromosome pair from either *G. stocksii* (E_1) or *G. anomalum* (B_1) to *G. hirsutum* $(AD)_1$.

The 4 F_1 hybrid plants were screened under natural conditions and by grafting (17 grafts). The hybrid remained resistant to CLCuV, a serious cotton disease. Hexaploid cotton had a higher fiber strength. Our results are similar to those reported by Liang et al. (2002) who developed hybrids of BC_2F_7 generation from the hexaploid of *G. hirsutum* x *G. stocksii* and found high fiber strength in their material. Singh and Bajaj (1996) crossed *G. herbaceum* with *G. stocksii* and found the most of the characters from *G. stocksii* were present in F_1. The transfer of characteristics of economic use (fiber strength and resistance to virus) from the wild species *G. stocksii* to the cultivated commercial cotton variety MNH-786 (*G. hirsutum*) is thus complete and to overcome the fiber length and lint percentage, the hexaploid will be exploited in back crossing with *G. hirsutum* to develop a CLCuV-resistant, good fiber strength and drought-resistant cotton variety in the future.

CONCLUSION

F_1 hexaploid plants of the cross between *G. stocksii* and *G. hirsutum* have exceptionally strong fibers and are resistant to cotton leaf curl virus disease, indicating the possibility of transferring fiber strength and cotton leaf curl virus disease-resistant genes from *G. stocksii* to *G. hirsutum* through hybridization. The findings further validate the method of introgressing desirable traits from diploid *Gossypium* species into tetraploid upland cotton.

ACKNOWLEDGMENTS

Research supported partially by the National Natural Science Foundation of China (#30571184 and #31271771) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

REFERENCES

Ahmad S, Mahmood K, Hanif M, Nazeer W, et al. (2011). Introgression of cotton leaf curl virus-resistant genes from Asiatic cotton (*Gossypium arboreum)* into upland cotton (*G. hirsutum*). *Genet. Mol. Res.* 10: 2404-2414.

Ahmad S, Noor-ul-Islam, Abid M, Wajad N, et al. (2010). Screening of cotton germplasm against Cotton Leaf Curl Virus. *Pak. J. Bot.* 42: 3327-3342.

Ahmed M, Saima I, Farhatullah and Aftab B (2011). Isolation and identification of differentially expressed genes between *Gossypium arboreum* and *Gossypium hirsutum* species. *African J. Biot.* 10: 337-343.

Amin KC (1940). Interspecific hybridization between Asiatic and new world cottons. *Ind. J. Agric. Sci.*404-412.

Ansari MAA (1958). Some records on *Gossypium stocksii*. *Pak. Cott.* 2: 27-32.

Arutyunova LG and Volkova LA (1987). Cytological characterization of triploid and hexaploid cotton hybrids. *Semenovodstva Kolapchanika* 20: 28-43.

Bao-Liang Z, Chen S, Xin-Lian S, Xiang-Gui Z, et al. (2003). Studies on the hybrid of *Gossypium hirsutum* L. and *Gossypium anomalum* L. *Act. Agron. Sin.* 29: 514-519.

Beaseley JO (1940). The production of polyploids in *Gossypium*. *J. Hered.* 31: 39-48.

Beaseley JO (1942). Meiotic chromosome behavior in species, species hybrids, haploid and induced polyploids of gossypium. *Genetics 27:25-54.*

Blank LM and Leathers CR (1963). Environmental and other factors influencing development of south western cotton rust. *Phytopathology* 53: 921-928.

Brinkerhoff LA (1970). Variation in *Xanthomonas malvacearum* and its relation to control. *Ann. Rev. Phytopathol.* 8: 85-110.

Brown MS and Menzel MY (1952). Polygenomic hybrids in *Gossypium* I. Cytology of hexaploids, pentaploids and hexaploid combinations. *Genetics* 37: 242-263.

Culp TW and Harrel DC (1973). Breeding methods for improving yield and fiber quality of Upland cotton (*G. hirsutum* L.). *Crop Sci.* 13: 686-689.

Culp TW and Green CC (1992). Performance of Obsolete and current cultivars and Pee Dee germplasm lines of cotton. *Crop Sci. 32: 35.* 32: 35-41.

Douwes H (1953). The cytological relationships of *Gossypium areysianum* Deflens. *J. Genet.* 51: 611.

Green CC and Culp TW (1990). Simultaneous improvement of yield, fiber quality and yarn strength in upland cotton. *Crop Sci.* 30: 66-69.

Hussain I (1995). A study of the Effects of Leaf Curl Virus on the Status of Minerals in Some Cotton Plants. Master's thesis, Department of Biochemistry, Bahauddin Zakariya University Multan.

Katterman FRH and Ergle DR (1970). A study of quantitative variations of nucleic acid in *Gossypium*. *Photochemistry* 9: 2007-2010.

Khan JA and J Ahmad (2005). Diagnosis, monitoring and transmission characters of *Cotton leaf curl virus*. *Curr. Sci.* 88: 1803-1809.

Khan NU, Abro HK, Kumbhar MB and Hassan (2001). Response of Upland Cotton Genotypes to Leaf Curl Virus (CLCuV). Proceedings of "3rd" National Conference of Plant Pathology, October 1-3, at NARC, Islamabad, 100-105.

Knight RL (1949). Distribution of Wild Species of *Gossypium* in Sudan. The Empire Cotton Growing Review.

Knight RL (1957). Black Arm Disease of Cotton and its Control. In The "Plant Protection Conference 1956" Butterworths Scientific Publications, London, 53-59.

Liang Z, Jiang R, Zhong W, He J, et al. (2002). Creation of the technique of interspecific hybridization for breeding in cotton. *Sci. China C Life Sci.* 45: 331-336.

Malik A and Sheikh AL (1974a). Interspecific hybrid of *Gossypium anomalum* Wawra et. Peyr. X *Gossypium stocksii* Mast. *J. Agri. Res.* 12: 171-174.

Malik A and Sheikh AL (1974b). Interspecific hybrid of *G. arboreum* L. and *G. stocksii* Mast. *J. Agri. Res.* 12: 241-244.

Phillips LL (1966). The cytology and phylogenetics of the diploid species of *Gossypium*. *Amer. J. Bot.* 53: 328-335.

Poisson C, Schwendiman J and Kammacher P (1969). Evidence of chromosomal homology between *G. anomalum* Wawra et. Peyer and *G. stocksii* Mast. *Cot. Et. Fiber Trop.* 24: 469-471.

Sacks EJ and Robinson AF (2009). Introgression of resistance to reniform nematode (*Rotylenchulus reniformis*) into upland cotton (*Gossypium hirsutum*) from *Gossypium arboreum* and a *G. hirsutum*/*Gossypium aridum* bridging line. *Field Crops Res.* 112: 1-6.

Saunders JH (1959). The wild species of Gossypium. Part II. The C and E genomes. The Empire Cotton Growing Review 36: 161.

Saunders JH (1961). The Wild Species of *Gossypium* and Their Evolutionary History. Oxford University Press, London.

Shepherd RL (1974). Transgressive segregation for root-knot nematodes resistance in Cotton. *Crop Sci.* 14: 872-875.

Singh GM and Bajaj YPS (1996). Hybridization between *Gossypium herbaceum* and *Gossypium stocksii* through embryo rescue. *Cell Res.* 6: 85-90.

Tariq M (2005). Status of cotton leaf curl virus in the Punjab. *Pakistan Cotton Growers* 9: 7-9.

Umbeck PF and Stewart JMcD (1985). Substitution of cotton cytoplasm from wild diploid species for cotton germplasm improvement. *Crop Sci.* 25: 1015-1019.