Pak. J. Bot., 8(1): 19—25, 1976.

INVESTIGATIONS INTO THE CAUSES OF UNPRODUCTIVITY OF OLD WORLD AND NEW WORLD COTTON HYBRIDS—2 (*GOSSYPIUM ARBOREUM*) X *GOSSYPIUM HIRSUTUM* AND 2 (*GOSSYPIUM ARBOREUM*) X *G. BARBADENSE.*

ANWAR MALIK

Cotton Research Institute, Multan, Pakistan.

Abstract

Tetraploids of *G. arboreum* synthesized by colchicine treatment were successfully utilized in crossing with *G. hirsutum* and *G. barbadense.* These tetraploids had low pollen fertility 53.8% but gigantic morphologically.

Hybrids with genomic constitution (C-4x *G. arboreum*) X *G. hirsutum*) and (C-4x *G. arboreum* X *G. barbadense*) were obtained successfully and studied cytologically. The backcross studies were undertaken but failure in this regard is due to some mechanical and genetic obstacles. The frequency of chromosomes association at the Metaphase-I was 13.41 I+18.55 II and 0.5 III and 14.2 I+18.1 II and 0.55 III respectively.

Introduction

The old world cottons are coined with a few resistant genes, thus it was thought worthwhile to transfer these to the commercial New world cottons. *Gossypium arboreum* (n = 13) was made tetraploid (n=26 by colchicine treatments. Hybrids were obtained in the combinations of 2 (*G. arboreum*) X *G. hirsutum* and 2 (*G. arboreum*) X *G. barbadense*. Quite contrary to our expectation, the hybrids did not produce any fruit throughout the season, attempts to produce fruit by selfing also resulted in failure. This unproductivity of the hybrids made us interested to investigate the nature of their meiosis and other cytological phenomenon. The present investigations, included the cytological and fertility studies of the colchicine induced tetraploid of *G. arboreum* 231-R, 2 (*G. arboreum*) X *G. hirsutum* and 2 (*G. arboreum*) X *G. barbadense.*

Materials and Methods

These studies were undertaken in the laboratories of Cotton Research Institute, Multan, during the years 1970-74. The species involved were:—

(i) *G arboreum* L. Var. 231-Rosac=and 119-sanguineum—(n=13)

(ii) *G. hirsutum* L. Var, Lassani-11 (n=26)

(iii) *G. barbadense* L. Var. Menufi (n=26)

Interspecific crosses were made by using the conventional method of soda straw technique for cotton. For meiotic studies flower buds of the plants under study were fixed in Carnoy's solution and usual Acetocarmine sqashes of pollen mother

cells (PMC's) were made. To obtain the tetraploids of old world cottons, seeds of *G. arboreum* var, 119-sanguineum and 231-Rosae were saoked in 0.03% and 0.05% colchicine solution for 6 hours before germination.

Review of Literature

Although extensive hybridization work has been done in the recent past among diploid and colchicine induced tetraploids of old world cottons and New world cottons only a few research workers have succeeded in getting the hybrids which could only survive upto the conset of buds/flowers. Zaitsav (1924, 1927) described in great detail the disturbed fructification in *G. hirsutum*, *G. barbadense* and *G. herbaceum* crosses and considered the formation of hybrid zygotes weak in development due to certain mechanical obstacles, and the artificial hybrids obtained were thought useless due to their complete sterility. Desai (1927) described a technique for crossing *herbaceum* female with *hirsutum* male. One hybrid plant obtained was successfully utilized as pollen parent in backcrossing with *G. hirsutum* and *G. herbaceum*, but without any cytological information.

Nakatomi (1931, 1940) described one polyploid plant of asiatic cotton (n=26) The F1 hybrids obtained, using New world cotton as female parent showed marked heterosis and complete sterility. Chromosome behaviour in meiotic division was quite irregular and the complete sterility of the F^1 hybrids were reported to depend on the formation of abortive germ cells.

Thankar et al (1936) obtained 19 seeds out of 4000 pollinations between old and new world cottons. Tanaka (1937) obtained some interesting results by ringing and wiring techniques where crosses between *herbaceum* X *hirsutum* produced hybrid seeds. Beasely (1940) observed that the use of C-4 X *G. arboreum* increased the crossability between it and *G. hirsutum*. Hutukaiti (1940) and Zhebrak (1940) obtained tetraploid plants of *G. arboreum* by colchicine treatment with low fertility and hybrid seeds by crossing it with *G. hirsutum* and produced amphidiploid plants/sectors by colchicine treatment to shoots, apices and seeds of *hirsutum* X *arboreum* crosses, respectively. Stephens (1940, 1942) reported a tetraploid plant of *G. arboreum* (n=26) obtained by colchicine treatment with 40—50% fertile female gametes, which readily crossed with New world (n=26) species.

Skovsted (1934) Iyengar (1944) reported auto-tetraploids of *G. arboreum* and *G. herbaceum* and crosses between C—4x *G. herbaceum* and cultivated Americans which survived upto flowering stage. Kasparyan (1945) obtained tetraploids of *G. arboreum* by means of colchicine which successfully crossed with *G. hirsutum*. Tominaga (1946) obtained tetraploids of 7 strains of Japanese cotton (n=13) with colchicine treatment showing gigas characteristics.

Patel et al (1940) reported a few fertile and chromosomally balanced back cross plants, with the genomic constitution of *hirsutum* X *arboreum* or *herbaceum* X *hirsutum*, which on further crossing gave 41% G.O.T. 1.15 inches fibre length and thrip resistance.

Gerstel (1953) and Kadir et al (1966) have reported the break down of the hybrid C-4x *G. arboreum* X *G. hirsutum*. Weaver (1957,58) ascribed the failure of hybrid formation between diploid *G. arboreum* as mother parent and *G. hirsutum*, to lack of

differentiation of embryo, and *G. arboreum* as male due to antagonism between the hybrid embryo and endosperm.

Arutjunova (1960) reported disturbed growth of such hybrid plants usually dying at some young stage, thought to be due to enzyme defficiency. However. normal growth was induced by grafting the hybrid on to *G. hirsutum* or *G. barbadense*. Efforts to overcome this sterility by vegetative reapproachment, ringing or pruning were not successful.

Result and Discussion

Seeds of *G. arboreum* varieties were soaked in 0.03% and 0.05% clochicine solution for 6 hours before germination. to obtain the tetraploid plants of these. A few plants were cytologically confirmed as tetraploids (Table 2), and utilized in present studies. The plants of colchicine treated *G. arboreum* were gigas in all the morphological characters, i.e. thick dark-green leaves, thick petals, more branching, larger bolls but low fertility etc. These were crossed and back crossed with *G. hirsutum* and *G. barbadense*, crossing and fertility data is presented in Table I.

Table 1. Crossing and fertility data.

Sr. No.	Name of cross	No. of Attempts	No. of bolls	Setting %	No of plants obtained	Viability %
1.	2 (*arboreum*) X *hirsutum*	1146	133	11.7	13	2.54
2.	2 (*arboreum*) X *barbadense*	395	7	1.77	1	1.61
3.	*hirsutum* X 2 (*arboreum*)	618	—	—	—	—
4.	*barbadense* X 2 (*arboreum*)	137	—	—	—	—
5.	*hirsutum* X 2 (*arboreum*) X *hirsutum*	201	—	—	—	—
6.	*hirsutum* X 2 (*arboreum*) X *barbadense*	687	—	—	—	—
7.	2 (*arboreum*) X *hirsutum* X *hirsutum*	211	—	—	—	—
8.	2 (*arboreum*) X *barbadense* X *hirsutum*	53	—	—	—	—

The synthetic tetraploid of *G. arboreum* is only fertile when used as female parent. and sterile as pollen parent, though its pollen stainability recorded was 53.8% (Table I). Similarly the hybrids of cross combination 1 and 2 were completely sterile though with 20.78% and 7.4% pollen stainability, and there hybrids could not review fertility even by backcrossing technique, and the hybrid plants obtained with full maturity are against the world opinion except Patel et al (1950) where he got a few fertile and chromosomally balanced backcross plants using *hirsutum* ans seed parent. In the present studies we could not get any hybrid by backcrossing with *hirsutum* and *barbadense*. both as seed and pollen parent as well (Table I).

For cytological studies more than 50 PMC's were studied at Metaphase-I in each case with varying number of clear plates, showing total number of 52 chromosomes. The frequency of chromosome association's at Metaphase-I of the plants under study is given in Table 2,3,4.

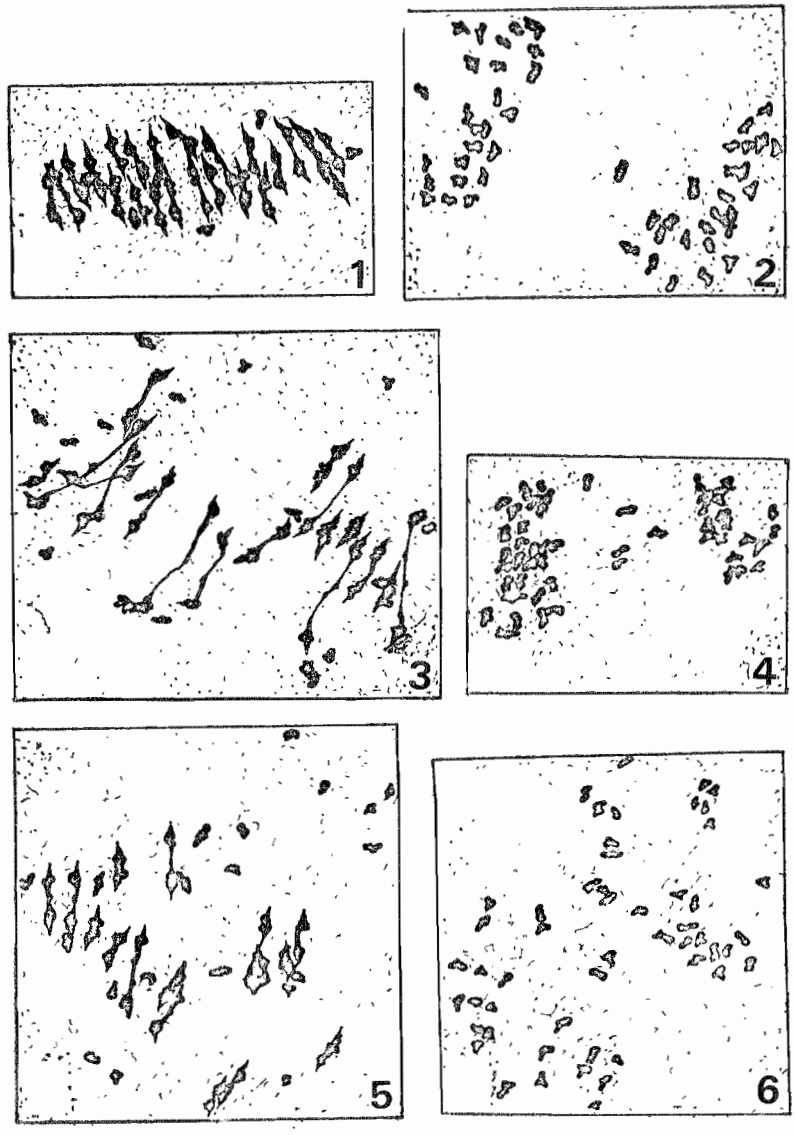
TABLE 2. Frequency of chromosome association's at Metaphase-I, C-^{4}x, *G. arboreum* L. Var. 231-Rosae.

Sr. No.	No. of PMC's	Valents I	II	III	Total	Remarks
1.	2	6	23	0	=52	A few laggards observed at Anaphase-I, The plant is fertile, with 53.8% pollen stainability.
2.	2	8	19	2	=52	
3.	8	9	20	1	=52	
4.	2	12	20	0	52	
5.	4	13	18	1	=52	
6.	2	17	16	1	=52	
Total:	20	Range : 6-17,	16-23	0-2		
		Average: 10.5	19.4	0.9		

Low fertility of the plants is revealed from the table which may be due to irregular meiotic behaviour. Beasely (1940), Nakatomi (1940), Hutukaiti (1940), Stephens (1942), Kasparyan (1945), Tominaga (1946), Gerstel (1953) and Kadir (1966) reported the autotetraploids of Asiatic cottons removing the barrier of crossability between Asiatic and New world cottons.

TABLE 3. Frequency of chromosome association's at Metaphase-I of the hybrid C-4x *G. arboreum* X *G. hirsutum*.

Sr. No.	No. of PMC's	I	II	III	Total	Remarks
1.	1	7	21	1	=52	At Metaphase-I, Univalent were scattered. At Sporad stage, cells with 3,4,5,6,7, & 8 sporads of 2.4%, 75.26%, 11.68%, 8.1, 1.72% and 0.34% respectively, were recorded 4-5 larggards were also recorded, at Anaphase-I. Plants sterile with 20.78% pollen stainability.
2.	3	9	20	1	=52	
3.	1	10	21	0	=52	
4.	2	11	19	1	+52	
5.	1	12	20	0	=52	
6.	3	13	18	1	=52	
7.	4	14	19	0	=52	
8.	3	16	18	0	=52	
9.	1	17	16	1	=52	
10.	1	18	17	0	=52	
11.	1	19	15	1	=52	
12.	1	20	16	0		
Total:	22	Range : 7-20	15-21	0-1		
		Average:13.41	18.55	0.5		



C—4x, *G. arboreum*.

Fig. 1. Metaphase-I showing 61+23 II

Fig. 2. Anaphase I showing one lagging chromosome.

C—1x, *G. arboreum* x *G. hirsutum*.

Fig. 3. Metaphase I showing 18 I+17 II.

Fig. 4. Anaphase I showing 4-lagging hromosomes.

C—4x. *G. arboreum* x *G. barbadense*.

Fig. 5. Mataphase I showing 20 I+16 II.

Fig. 6. Anaphase I, showing 6 lagging chromosomes.

The 13 plants obtained of this combination were morphologically alike, and completely sterile (Table-II). This sterility can be attributed to the large number of univalents and few multivalents fromation at Metaphase-I. Besides this the stigma of the flowers were usually inverted causing self compatibility. These plants showed a marked heterosis but are completely sterile. Gerstel (1953), Kadir (1966) and Hu ukaiti (1940) reported the break down of hybrids of this combination.

TABLE 4. Frequency of chromosome association's at Metaphase-I, of the hybrid C-4x *G. arboreum* X *G. barbadense*.

Sr. No.	No. of PMC's	Valents I	II	III	Total	Remarks
1.	3	10	21	0	=52	At Metaphase-I, Univa-
2.	1	11	19	1	=52	lents were scattered, and at
3.	1	13	18	1	=52	Anaphase-I, 4-6 laggards
4.	1	14	16	2	=52	were observed at sporad
5.	1	14	19	0	=52	stage, cell with 2,4,5,6 and
6.	1	15	17	1	=52	7 sporads with 0.8%, 84.0%
7.	1	17	16	1	=52	10.4%, 3.2% and 1.8%
8.	1	20	16	0	=52	were recorded. Pollen stain-
9.	1	22	15	0	=52	ability was 7.4% and the plant is completely sterile.
Total:	11	Range :10-22	15-21	0-2		
		Average:14.2	18.1	0.55		

Only one plant survived, which was completely sterile. (Table-I). This sterility can also be attributed to the irregular chromosome behaviour at meiotic division. Besides this the stigma of the flowers were usually inverted or coiled, causing self compatibility. Desai (1927) and Nakatomi (1940) claimed one hybrid plant between *herbaceum* female and *hirsutum* male and successfully backcrossed with both the parents, this difference may be due to the species or varieties used. The only successful backcross reported by Patel et al (1950) and Desai (1927) was between (*hirsutum* X *arboreum* or *herbaceum* X *hirsutum*) having desirable fibre characters and thrip resistance.

Weaver (1957,58) Zaitsev (1924, 1927), Desai (1927), Thaker et al (1936) Tanaka (1937) and Arutjunova (1960) crossed the asiatic cotton at diploid level (n=13) and attributed different opinions for the failure to get hybrids or break down like antagonism between hybrid embryos and endosperms, enzymes deficiency, mechanical obstacles and assumed that this may be removed by use of synthetic tetraploids of old world cottons. In the present studies hybrids of both combination were obtained easily but could not survive upto maturity except one plant of C-4x, *G. arboreum* X *G. barbadense* in the first instance. However, efforts were continued by using grafting technique, which did not prove good in the sense that the grafted and ungrafted hybrid plants survived and utilized in the the present studies at the latter stage.

Acknowledgements

The authors feel greatly indebted to Dr. Mahbub Ali, Director, Cotton Research Institute, Multan for his able guidance and valuable suggestions during the course of these studies.

References

Arutjunova. L.G. 1960. Interspecific hybridization in genus *Gossypium* L. Plant. Br. Abst. **32:** 5047.

Beasley, J.O. 1940. Hybridization of American 26-chromosome and asiatic 13— chromosome species of *Gossypium*. J. Agric. Res., **60:** 175-182.

Desai, B.B. 1927. A cross between Indian and American cottons. Ind. J. Agric. J., **22:** 351-353.

Hutukaiti, S. 1940. 1940. On tetraploid Asiatic cotton and its characteristics. Bot & Zool. **8:** 597-601 (Japanese).

Iyengar, N. . 1944-a. Cytological investigations on auto, allo-tetraploid Asiatic cottons. Indian. J. Agric. Sci., **14:** 30-40.

Kasparyan, A.S. 1945. Overcoming non-crossability in cotton. C.R. (Docklady) Acad. Sci. O.R.S.S., **49:** 66-68.

Kadir, Z.B.A., Khan. M. and A.S. Islam. 1966. Attempts to overcome crossability barrier between *Gossypium* species. *I.G. arboreum* X *G. hirsutum*. Sind. Uni. Res. J. Sci. Ser., **2:** 42-46.

Nakatomi, S. 1931. Hybridization between old world and New World cotton species and the the chromosome behavicur of the pollen mother cells in the F1 hybrids. Jap. J. Bot., **5:** 371-384.

————————. 1940. Induced poly-ploidy in asiatic varieties of cotton plant by colchicine treatment Proc. crop. sci. Soc. Japan., **12:** 16-20.

Patel, G.B, and B.J. Thakar, 1950. Cotton improvement through interespecific hybridization. Indian Cott. Gr. Rev., **4:** 185-198.

Stephens, S.G. 1940. Colchicine treatments as a means of inducing polyploidy in cotton. Trop. Agric. Trin., **17:** 23-25.

————————. 1942. Colchicine induced polyploids in *Gossypium*. I. An autotetraploid asiatic cotton and certain of its hybrids with wild diploid species. J. Genet., **44:** 272-295.

Skovsted, A. 1934. Cytolgocial studies in cotton. II-Two interspecific hybrids between Asiatic and New world cottons. J. Genet., **28:** 407-424.

Thakar, B.J. and K.C. Amin. 1936. Interspecific hybridization in cotton Poona. Agric. Coll. Mag., **28:** 19-20.

Tanaka, M. 1937. The application of ringing and wiring to interespecific crosses of the genus *Gossypium*. Mems. Coll. Agric. Koyoto, No. 39.

Tominaga. Y. 1946. On artificially produced tetraploids of cotton. Jap. J. Genet., **21:** 60-61.

Wever, J.B. 1957. Embryolegical studies following interspecific crosses in *Gossypiam*. I. *G. hirsutum* X *G. arboreum*. Plant. Br. Abst. **27:** 694.

————————. 1958. Embryological studies following interspecific crosses in *Gossypium*. II-*G. arboreum* X *G. hirsutum*. Plant. Br. Abst. **28:** 792.

Zlebrak, A.R. and M.M. Rzaev. 1940. Mass production of amphiploids by colchicine treatments in cotton. C.R. (Docklady) Acad. Sc. U.S.S.R. **26:** 159-62.

Zaits, G.S. 1924. On the fructification in interespecific hybrids of cotton. Trans. Turkestan. Pl. Br. Sta. Tashkent. No. 2 (Russian).

————————. 1927. Artificial and natural Asiatic-American cotton hybrids. Ind. Agri. J. **22:** 155-167 and 261-268.