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## CYTOGENETIC ANALYSIS OF COTTON HYBRIDS DERIVED FROM INTROGRESSIVE LINES

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### SUMMARY

Cotton is one of the world's most important natural fiber and cash crops. The research carried out studies of plants F<sub>1</sub>-F<sub>4</sub> considering the importance of cytogenetic analysis of interspecific hybrids for identifying structural differences between homologous chromosomes of crossed forms and substitution of individual chromosomes or chromosome segments because of introgression of interspecific hybrids in cotton breeding. The article comprised a cytogenetic analysis of introgressive lines obtained through the participation of intergenomic crosses and F<sub>1</sub>-F<sub>4</sub> cotton hybrids. The results revealed that in crossed variants of F<sub>1</sub>-F<sub>4</sub> hybrid plants, the presence of open bivalents and univalents in PMC (pollen mother cell) showed the absence of complete conjugation in the chromosomes. It could be due to the structural differences between the homologous chromosomes in the crossed forms caused by exchanging chromosomes with alien ones. According to the tetrad analysis, the average value ranged from 95.65% F<sub>1</sub>L-158/16 × Sultan to 99.61% F<sub>1</sub>L-4747-48/16 × Sultan in F<sub>1</sub> hybrids. Based on the tetrad analysis in 16 combinations, the meiotic index ranged from 96.76 ± 0.34 to 99.54 ± 0.19 in F<sub>2</sub> hybrids and 96.51 ± 0.56 to 99.34 ± 0.30 in F<sub>3</sub> hybrids, and in 17 combinations, the range was from 97.14 ± 0.29 to 98.92 ± 0.12 in F<sub>4</sub> hybrids. It also confirmed that meiosis is preceding naturally in the remaining hybrid variants, with a decrease observed in the meiotic index. The results also increased the number of other types of gametes (Monod, dyad, triad, pentad, hexad, and polyad), negatively affecting normal gametes formation.

**Keywords:** Upland cotton, *G. barbadense* L., *G. hirsutum* L., hybrids, introgressive lines, meiosis, tetrad, sporadic, bivalent, degree of fecundity

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**Key findings:** The cytological analyses of introgressive cotton lines and hybrids confirmed their donor ability to enrich the cotton genome. Results established the stabilization of the meiotic division that raised the meiotic index and pollen fertility. The structural differences between homeologous chromosomes confirm the introgressed fragments of wild forms and valuable traits in the cultivated cotton genomes.

## INTRODUCTION

Cotton is one of the most economically crucial fiber crops worldwide (Wu *et al.*, 2017; Aslam *et al.*, 2020). The genus *Gossypium* L. contains about 53 species, where the cultivated four comprised two diploid and two allotetraploid (Wendel and Grover, 2015; Yin *et al.*, 2020). However, recent studies reported developing and describing some new species (Stewart *et al.*, 2015; Gallagher *et al.*, 2017). Divergence analysis based on DNA molecular markers enunciated that the main diploid branches of the cotton genus diverged about 7–11 million years ago (Wendel and Grover, 2015; Chen *et al.*, 2016, 2017a).

Subsequently, the cotton ancestors diversified into ~46 diploid species (divided into eight genome groups designated as A–G and K) and seven allotetraploid species designated as AD genomes (Abdurakhmonov *et al.*, 2008; Wendel and Grover, 2015; Grover *et al.*, 2015; Wu *et al.*, 2017; Shim *et al.*, 2018). In general, the polyploid fiber appeared about 1–2 million years ago, probably due to transoceanic dispersal events involving an African-Asian A-genome type that later hybridized with a New World D-genome type (Wendel and Grover, 2015; Chen *et al.*, 2017a, b).

In the present era, scientific research on developing cotton by utilizing the existing cotton gene pool in cotton-growing countries, specifically increasing productivity and improving fiber quality, has not lost its relevance (Abdurakhmonov *et al.*, 2014). It also requires strengthening research on identifying the unique donors with valuable traits for the economy and involving them in the selection process by introducing wild cotton accessions from the gene pool of Uzbekistan. Previous studies on interspecific 3-4-5 specific hybridization (*G.hirsutum* L., *G.barbadense* L., *G.thurberi* Tod., *G.raimondii* Ulbr. and

*G.arboreum* L.) have developed new introgression cotton lines and cultivars that can serve as a base material in breeding for practical and genetic selection in cotton (Sherimbetov *et al.*, 2020; Eschanov and Namazov, 2021; Anwar *et al.*, 2023; Muminov *et al.*, 2023; Namazov *et al.*, 2023). Some researches of Uzbek scientists are geared toward genetically and geographically remote hybridization (Kholmurodova *et al.*, 2023; Namazov *et al.*, 2023) and developing cotton progenies with none toxic enantiomer, i.e., high level of (+) gossypol (Uzbekov *et al.*, 2012; Vshivkova *et al.*, 2012).

The wild and semi-wild types of cotton are well-known to belong to diverse genomes, which are also complex to cross with cultivated cultivars, and the fact that most of the interspecies hybrids obtained with them showed the symptoms of sterility and non-fecundity led to their infrequent use in practical breeding (Zhang *et al.*, 2014; Sanamyan *et al.*, 2022). However, great attention has focused on studying the causes of sterility and non-fecundity in interspecies hybridization. In particular, as a result of studying the development of the reproductive circle of wild cotton intraspecific and interspecific hybrids, the interspecific hybrids were full-fertile and developed well (Percy *et al.*, 2014; Miyazaki *et al.*, 2017; Konan *et al.*, 2020). The development of the sexual sphere, that is, the process of meiosis, has passed without any disturbances. However, the study of the progress of the hybrid sexual circle between the species, *G. hirsutum* L. × *G. Trilobum*, exhibited several disorders in the course of meiosis, micro- and macro-sporogenesis, and gametophytes development, authenticating significant pollen and seed shoot sterility (Egamberdieva, 2017; Panda *et al.*, 2023).

Therefore, a conclusion finds that it is imperative to study the genetic and cytological aspects of cotton breeding material with a

complex genetic basis to ensure stabilizing the various economic traits in higher generations based on the achievements of modern science. The present task sought to develop the primary material that is genetically stable, incorporating valuable economic features, such as early maturity, high yield, better fiber quality, and tolerance to specific biotic factors. The study also included exploring cytogenetic parameters by introducing genetically enriched introgression lines developed in the laboratory through various complex hybridization methods into crosses from previous study years.

## MATERIAL AND METHODS

### Plant material

The research commenced in the crop seasons 2016 until 2020 at the Scientific Research Institute of Cotton Breeding, Seed Production and Agrotechnologies, Tashkent, Uzbekistan. Research samples used were 17 introgression lines of upland cotton (*Gossypium hirsutum* L.), i.e., L-4672-73/16 × Sultan, L-4674-77/16 × Sultan, L-4679-81/16 × Sultan, L-4684-86/16 × Sultan, L-138/16 × Sultan, L-470/1/16 × Sultan, L-95/16 × Sultan, L-158/16 × Sultan, L-200/16 × Sultan, L-MVG/16 × Sultan, L-58/16 × Sultan, L-1979/16 × Sultan, L-175/248/16 × Sultan, L-12/06/16 × Sultan, L-4747-48/16 × Sultan, L-BSG/16 × Sultan, and L-588/16 × Sultan, developed during of past years in the Laboratory of Cotton Genetics and Cytology.

### Cytogenetical analysis

The two different analyses, including a) Analysis of the pollen fecundity and b) Analysis of meiosis in metaphase-I and tetrad stage, have been performed in cytogenetic studies. In the flowering stage, picking three flowers from the plants of each genotype every morning had their pollen fecundity analysis performed. In this analysis, temporarily crumpled preparations from the accessions under laboratory conditions used a 2% acetocormin solution. After placing the preparations in a Petri dish, it is necessary to put them in the

refrigerator for one day to ensure better staining. Each preparation's analysis was according to ten fields of view.

In the cytological analysis of meiosis, collecting twice a week of young shoots (2-4 mm) from each studied genotype plants sustained fixing in acetoalcohol solution (3:7) (Sanamyan and Musaev, 1990). In the sporad stage analysis, assessing several cotton plant buds from each hybrid plant variant had their meiotic index (M) calculated, specifically a cytogenetic study of the normal percentage of tetrads relative to the total number of sporads using the following:

$$Mi = \frac{II}{N} \times 100 \%$$

Where

II = number of normal tetrads

N = total number of sporads

### Statistical analysis

Cytogenetic studies underwent further analysis with the help of the 'Sporada' and 'Pollen' programs, with the processing carried out in the large and small accessions of statistical indicators by following the methodology of Dospekhov (1985).

## RESULTS

Measuring the similarities and differences in cotton types and species involved in hybridization can proceed by the number of conjugating chromosomes in the metaphase-I stage of meiosis. However, with the accumulation of new data, it became more apparent that conjugation at the metaphase-I stage of meiosis does not completely reflect the process of chromosome pairing, with a discrepancy in the number of chromosomes at the early pachytene and diplotene stages in wheat (Gill, 2015; Darrier *et al.*, 2022). Therefore, the said study proposed the total absence of chromosome conjugation in the prophase of meiosis as 'asynapsis,' and the appearance of unpaired chromosomes in the late stages of meiosis, when there was

conjugation in the pachytene phase of meiosis, as 'desynapsis.'

In separating hybrid genotypes with different types of karyotype deviations in the initial generations, the meiotic division of several complex interspecific hybrids has been studied (Sanamyan and Rakhmatullina 2003; Sanamyan and Bobokhujaev, 2019). Such a result was positive because the studied material reached intensive use for selection in numerous breeding processes. However, the presence of some deviations in the karyotype of the initial hybrids would have been of negative importance for selection in cotton.

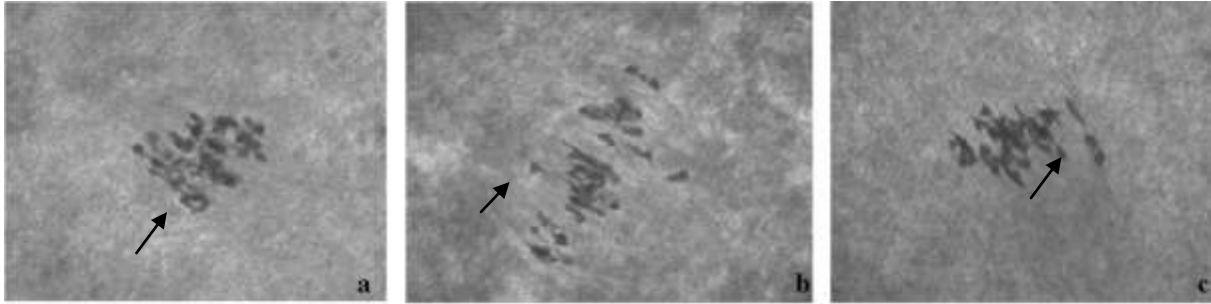
For cytological characterization of  $F_1$  hybrids obtained from crossing the introgression cotton lines, the buds of the hybrid plants acquired from 17 crossing options gained fixing. However, the analysis of chromosome pairing at the metaphase-I stage of meiosis proceeded only in 10 crossing combinations, as no buds appeared at this stage in seven crossing combinations. Four  $F_1$  hybrid variants, viz., L-4684-86/16 × Sultan, L-158/16 × Sultan, L-1979/16 × Sultan, and L-588/16 × Sultan, showed the highest meiotic stability, in which 26 normally closed bivalents emerged at the metaphase-1 stage of meiosis, with other associations of chromosomes were

undetected (Table 1). However, in the remaining six crossing variants, different deviation types were evident in some pollen mother cells (PMC) at the metaphase-I stage of meiosis. A single quadrivalent pairing of chromosomes was visible in two PMCs in one  $F_1$  hybrid L-4672-73/16 × Sultan (Figure 1a). The remaining eight of the 42 PMCs showed normal closed bivalents and a small number of double univalents and open bivalents. The formation of a quadrivalent association of chromosomes in hybrid plants of the above hybridization combinations also indicates the presence of a translocation in the differentiation of chromosome sets in parental forms.

The presence of open bivalents and univalent chromosomes also indicated the occurring hidden structural changes in chromosomes of the inter-breeding types. In addition, three more hybrid variants showed having double univalents in the PMC. However, in the PMC of two hybrid variants  $F_1$  L-138/16 × Sultan and  $F_1$ L-MVG/16 × Sul-ton along with normal bivalents, up to four univalent chromosomes were also prominent (Figure 1b). In another variant,  $F_1$ L-58/16 × Sul-ton, 14 univalents were evident. The chromosomes of the interbreeding forms were sufficiently

**Table 1.** Analysis of chromosomes conjugation at the metaphase-I stage of meiosis in  $F_1$  hybrids.

Plant material	Average number of cells			
	Univalent	Bivalent	Open	Quadrivalent
$F_1$ L-4672-73/16 × Sultan	0.25±0.24	25.38±0.59	-	0.25±0.24
$F_1$ L-4674-77/16 × Sultan	-	-	-	-
$F_1$ L-4679-81/16 × Sultan	-	-	-	-
$F_1$ L-4684-86/16 × Sultan	-	26.00±0.00	-	-
$F_1$ L-138/16 × Sultan	0.18±0.17	25.91±0.09	-	-
$F_1$ L-470/1/16 × Sultan	-	-	-	-
$F_1$ L-95/16 × Sultan	-	-	-	-
$F_1$ L-158/16 × Sultan	-	26.00±0.00	-	-
$F_1$ L-200/16 × Sultan	-	-	-	-
$F_1$ L-MVG/16 × Sultan	0.50±0.25	25.50±0.14	0.25±0.13	-
$F_1$ L-58/16 × Sultan	0.93±0.90	25.53±0.45	-	-
$F_1$ L-1979/16 × Sultan	-	26.00±0.00	-	-
$F_1$ L-175/248/16 × Sultan	-	-	-	-
$F_1$ L-12/06/16 × Sultan	-	-	-	-
$F_1$ L-4747-48/16 × Sultan	-	25.91±0.09	0.09±0.09	-
$F_1$ L-BSG/16 × Sultan	-	25.91±0.09	0.09±0.09	-
$F_1$ L-588/16 × Sultan	-	26.00±0.00	-	-



**Figure 1.** Configuration of chromosomes at the metaphase-I stage of meiosis in F<sub>1</sub> hybrids: a) F<sub>1</sub>L-4672-73/16 × Sultan: 26 bivalents, one quadrivalent; b) F<sub>1</sub>L-138/16 × Sultan: 26 bivalents, of which two are open bivalents; c) F<sub>1</sub>L-158/16 × Sultan: 26 bivalents (open bivalents are indicated with an arrow).

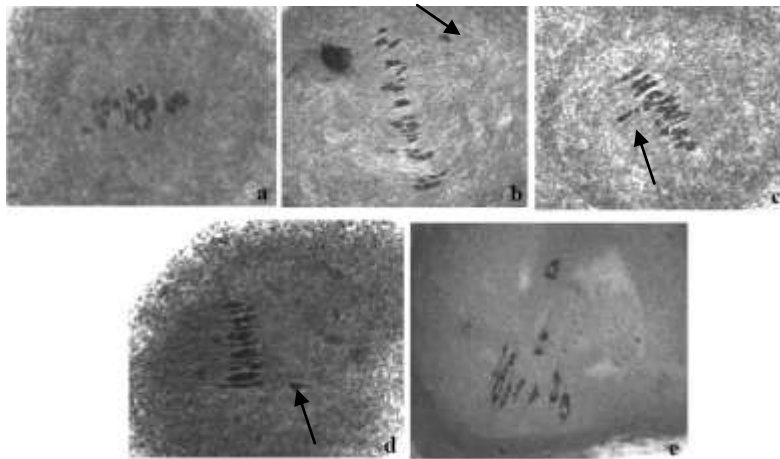
homologous for the conjugation in the prophase of meiosis; however, the hidden structural variations led to premature separation of chromosomes and the appearance of ‘pseudo univalents’ in the metaphase-I stage of meiosis. Two other F<sub>1</sub> hybrid variants, i.e., L-4747-48/16 × Sultan and L-BSG/16 × Sultan, also had the presence of normally closed bivalents and very few open bivalents in their PMC.

Fixing the buds from plants of 17 hybridization options helped study the cytological characteristics of F<sub>2</sub> hybrids. The

analysis of chromosome pairing at the metaphase-I stage of meiosis also transpired only in nine crossing combinations, whereas, in the remaining eight crossing combinations, no buds were available at the metaphase-I stage of meiosis. In three F<sub>2</sub> hybrids, i.e., L-4674-77/16 × Sultan, L-95/16 × Sultan, and L-58/16 × Sultan, the standard closed bivalents formation ensued at the metaphase-I stage of meiosis and showed the highest meiotic stability, while not detecting other association of chromosomes (Table 2).

**Table 2.** Analysis of chromosomes conjugation at the metaphase-I stage of meiosis in F<sub>2</sub> hybrids.

Plant material	Average number of cells			
	Univalent	Bivalent	Open	Quadrivalent
F <sub>2</sub> L-4672-73/16 × Sultan	0.10±0.10	25.70±0.20	0.20±0.19	-
F <sub>2</sub> L-4674-77/16 × Sultan	-	26.00±0.00	-	-
F <sub>2</sub> L-4679-81/16 × Sultan	-	-	-	-
F <sub>2</sub> L-4684-86/16 × Sultan	0.09±0.09	25.82±0.09	0.09±0.09	-
F <sub>2</sub> L-138/16 × Sultan	0.08±0.07	25.85±0.10	0.08±0.07	-
F <sub>2</sub> L-470/1/16 × Sultan	0.31±0.13	25.92±0.07	0.08±0.07	-
F <sub>2</sub> L-95/16 × Sultan	-	26.00±0.00	-	-
F <sub>2</sub> L-158/16 × Sultan	-	-	-	-
F <sub>2</sub> L-200/16 × Sultan	0.10±0.10	25.80±0.13	0.10±0.10	-
F <sub>2</sub> L-MVG/16 × Sultan	-	-	-	-
F <sub>2</sub> L-58/16 × Sultan	-	26.00±0.00	-	-
F <sub>2</sub> L-1979/16 × Sultan	-	-	-	-
F <sub>2</sub> L-175/248/16 × Sultan	-	-	-	-
F <sub>2</sub> L-12/06/16 × Sultan	-	-	-	-
F <sub>2</sub> L-4747-48/16 × Sultan	-	-	-	-
F <sub>2</sub> L-BSG/16 × Sultan	-	-	-	-
F <sub>2</sub> L-588/16 × Sultan	0.13±0.13	25.75±0.16	0.13±0.13	-



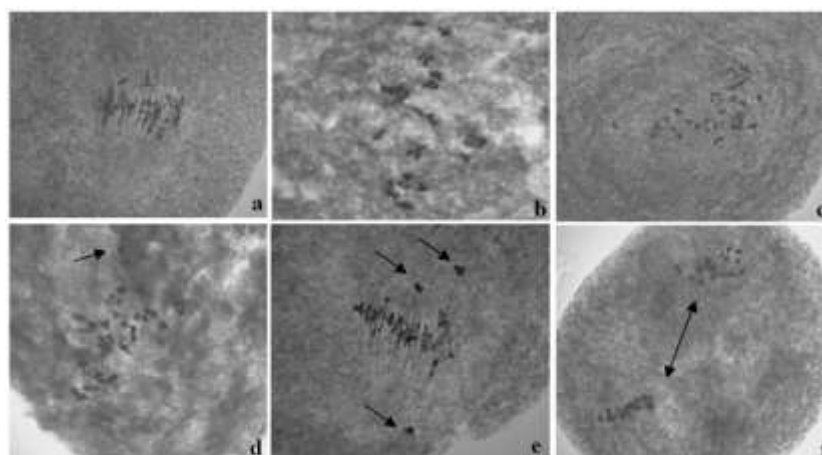
**Figure 2.** Chromosomes configuration of metaphase-1 phase of meiosis in hybrids  $F_2$ : a) in  $F_2L-138/16 \times$  Sultan hybrid 26 normal bivalent; b) in  $F_2L-470/1/16 \times$  Sultan hybrid 26 normal bivalent; c) in  $F_2L-200/16 \times$  Sultan hybrid 26 normal bivalent, of which one is open bivalent; d) in  $F_2L-588/16 \times$  Sultan hybrid 26 normal bivalent, of which one is open bivalent; e) in  $F_2L-12/06/16 \times$  Sultan hybrid incomplete PMCs bivalents (open bivalents are indicated with an arrow).

However, in the remaining six studied hybrids, i.e.,  $F_2L-4672-73/16 \times$  Sultan,  $F_2L-4684-86/16 \times$  Sultan,  $F_2L-138/16 \times$  Sultan,  $F_2L-470/1/16 \times$  Sultan,  $F_2L-200/16 \times$  Sultan, and  $F_2L-588/16 \times$  Sultan, very few open bivalents were remarkable (Figures 2c, d, e) along with normal closed bivalents at the metaphase-1 stage of meiosis in pollen mother cells (PMC) (Figures 2a, b). In  $F_2$  hybrids of six crossing variants, the open bivalents in PMC indicated incomplete conjugation in chromosomes due to structural differences between the chromosome sets of the crossing types.

At the metaphase-I stage of meiosis, the analysis of chromosome pairing in complex interspecific  $F_3$  hybrids ensued in 10 hybrid combinations. Meanwhile, no buds occurred at the metaphase-I stage of meiosis in the remaining seven cross combinations. Four hybrids, i.e.,  $F_3L-4672-73/16 \times$  Sultan,  $F_3L-470/1/16 \times$  Sultan,  $F_3L-1979/16 \times$  Sultan, and  $F_3L-4747-48/16 \times$  Sultan displayed the highest meiotic stability, in which 26 normal closed bivalents underwent formation at the metaphase-I stage of meiosis, not detecting any other chromosomes association (Figures 3a, b, c).

Observations in five hybrid variants, viz.,  $F_3L-4679-81/16 \times$  Sultan,  $F_3L-4684-86/16 \times$  Sultan,  $F_3L-200/16 \times$  Sultan,  $F_3L-58/16 \times$  Sultan, and  $F_3L-175/248/16 \times$  Sultan, included the normally closed bivalents (from  $25.33 \pm 0.29$  to  $25.69 \pm 0.29$ , on average per cell) and very few open bivalents (0, from  $23 \pm 0.22$  to  $0.63 \pm 0.59$ , on average per cell) at the metaphase-I stage of meiosis in separate PMCs (Figures 3d, e, f). In addition, in two crossing variants,  $F_3L-175/248/16 \times$  Sultan and  $F_3L-BSG/16 \times$  Sultan, the separate univalents were evident in maternal cells of the pollen ( $0.58 \pm 0.28$  and  $1.00 \pm 0.50$ , on average per cell, respectively) (Table 3). The absence of complete conjugation in the chromosomes emerged due to open bivalents in the PMC of the  $F_3$  hybrid plants of five crossing variants. Likewise, the presence of univalent chromosomes apart in two crossing variants appeared due to the structural differences between homologous chromosomes in the crossing forms caused by exchanging chromosomes with alien ones.

For cytological characteristics of  $F_4$  hybrids, fixation of the buds obtained from plants of 17 hybrids surfaced. Analysis of chromosome pairing at the metaphase-I stage



**Figure 3.** Configuration of metaphase-I of meiosis in F<sub>3</sub> hybrids: a) F<sub>3</sub>L-470/1/16 × Sultan 26 normal bivalents; b and c) F<sub>3</sub>L-1979/16 × Sultan hybrid 26 bivalent; d) in F<sub>3</sub>L-4679-81/16 × Sultan hybrid 24 normal and two open bivalents; e and f) in F<sub>3</sub>L-200/16 × Sultan hybrid 23 normal and three open bivalents, plate of three bivalents are located below; f) in chromosomes inadequate division of meiosis metaphase-II.

**Table 3.** Analysis of chromosomes conjugation at the metaphase-I stage of meiosis in F<sub>3</sub> hybrids.

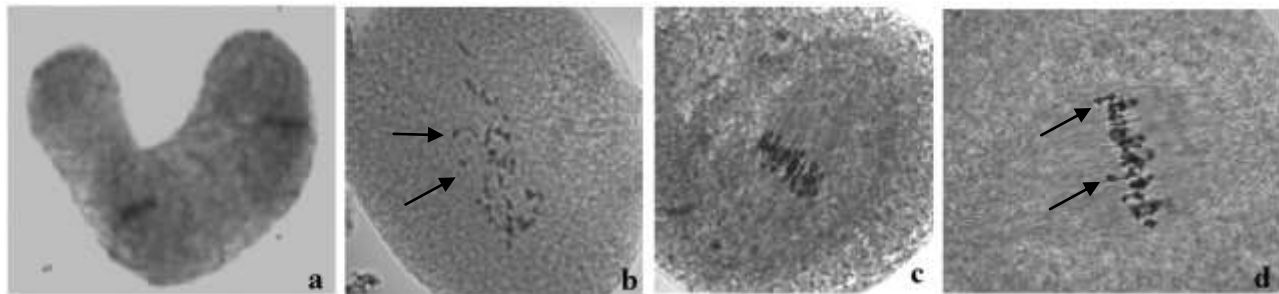
Plant material	Average number of cells			
	Univalent	Bivalent		Quadrivalent
		Close	Open	
F <sub>3</sub> L-4672-73/16 × Sultan	-	26.00±0.00	-	-
F <sub>3</sub> L-4674-77/16 × Sultan	-	-	-	-
F <sub>3</sub> L-4679-81/16 × Sultan	-	25.77±0.22	0.23±0.22	-
F <sub>3</sub> L-4684-86/16 × Sultan	-	25.69±0.30	0.31±0.30	-
F <sub>3</sub> L-138/16 × Sultan	-	-	-	-
F <sub>3</sub> L-470/1/16 × Sultan	-	26.00±0.00	-	-
F <sub>3</sub> L-95/16 × Sultan	-	-	-	-
F <sub>3</sub> L-158/16 × Sultan	-	-	-	-
F <sub>3</sub> L-200/16 × Sultan	-	25.38±0.59	0.63±0.59	-
F <sub>3</sub> L-MVG/16 × Sultan	-	-	-	-
F <sub>3</sub> L-58/16 × Sultan	-	25.60±0.39	0.40±0.39	-
F <sub>3</sub> L-1979/16 × Sultan	-	26.00±0.00	-	-
F <sub>3</sub> L-175/248/16 × Sultan	0.58±0.28	25.67±0.14	0.25±0.13	-
F <sub>3</sub> L-12/06/16 × Sultan	-	-	-	-
F <sub>3</sub> L-4747-48/16 × Sultan	-	26.00±0.00	-	-
F <sub>3</sub> L-BSG/16 × Sultan	1.00±0.50	25.33±0.29	-	-
F <sub>3</sub> L-588/16 × Sultan	-	-	-	-

of meiosis progressed in 11 hybrids, and no bud arose at the metaphase-I stage of meiosis in the remaining six crossing combinations. Four crossing variants of F<sub>4</sub> hybrids, i.e., F<sub>4</sub>L-4684-86/16 × Sultan, F<sub>4</sub>L-95/16 × Sultan, F<sub>4</sub>L-4747-48/ × Sultan, and F<sub>4</sub>L-588/16 × Sultan were also descriptive of the highest meiotic stability. The formation of 26 normally closed

bivalents occurred at their metaphase-I stage of meiosis, with no other chromosome association recognized (Table 4). In three hybrids, i.e., F<sub>4</sub>L-4679-81/16 × Sultan, F<sub>4</sub>L-1979/16 × Sultan, and F<sub>4</sub>L-12/06/16 × Sultan during the metaphase-I stage of meiosis, very few open bivalents (ranging from 0.17 ± 0.15 to 0.50 ± 0.35, on average per

**Table 4.** Analysis of chromosomes conjugation at metaphase-I stage of meiosis in F<sub>4</sub> hybrids.

Plant material	Average number of cells			
	Univalent	Bivalent		Quadrivalent
		Closed	Open	
F <sub>4</sub> L-4672-73/16 × Sultan	-	-	-	-
F <sub>4</sub> L-4674-77/16 × Sultan	0.14±0.13	25.86±0.13	0	0
F <sub>4</sub> L-4679-81/16 × Sultan	0	25.83±0.15	0.17±0.15	0
F <sub>4</sub> L-4684-86/16 × Sultan	0	26.00±0.00	0	0
F <sub>4</sub> L-138/16 × Sultan	-	-	-	-
F <sub>4</sub> L-470/1/16 × Sultan	-	-	-	-
F <sub>4</sub> L-95/16 × Sultan	0	26.00±0.00	0	0
F <sub>4</sub> L-158/16 × Sultan	0.50±0.47	25.63±0.25	0.25±0.24	0
F <sub>4</sub> L-200/16 × Sultan	0.06±0.06	25.69±0.15	0.25±0.14	0
F <sub>4</sub> L-MVG/16 × Sultan	-	-	-	-
F <sub>4</sub> L-58/16 × Sultan	-	-	-	-
F <sub>4</sub> L-1979/16 × Sultan	0	25.67±0.29	0.33±0.29	0
F <sub>4</sub> L-175/248/16 × Sultan	-	-	-	-
F <sub>4</sub> L-12/06/16 × Sultan	0	25.50±0.35	0.50±0.35	0
F <sub>4</sub> L-4747-48/16 × Sultan	0	26.00±0.00	0	0
F <sub>4</sub> L-BSG/16 × Sultan	0.10±0.11	25.60±0.23	0.30±0.22	0
F <sub>4</sub> L-588/16 × Sultan	0	26.00±0.00	0	0



**Figure 4.** Configuration of metaphase-I of meiosis in F<sub>4</sub> hybrids of cotton: a) F<sub>4</sub>L-4672-73/16 × Sultan hybrid abnormal anaphase-I; b and c) F<sub>4</sub>L-158/16 × Sultan hybrid b) 24 normal bivalent and four univalent; c) 26 normal bivalent; d) F<sub>4</sub>L-MVG/16 × Sultan hybrid 24 normal bivalent and two open bivalent.

cell) came out (Figure 4a). Similarly, normally closed bivalents (ranging from  $25.83 \pm 0.15$  to  $25.50 \pm 0.35$ , on average per cell) were notable (Figures 4b, c).

Additionally, in four cross combinations, i.e., F<sub>4</sub> L-4674-77/16 × Sultan, F<sub>4</sub>L-158/16 × Sultan, F<sub>4</sub>L-200/16 × Sultan, and F<sub>4</sub>L-BSG/16 × Sultan, different univalents were prevalent in separate PMCs (from  $0.06 \pm 0.06$  and  $0.50 \pm 0.47$ , on average per cell) (Figures 4b, c, d). In some PMCs of three

hybrids, viz., F<sub>4</sub>L-158/16 × Sultan, F<sub>4</sub>L-200/16 × Sultan, and F<sub>4</sub>L-BSG/16 × Sultan, the univalents and open bivalents had simultaneous formation. In chromosomes, the absence of complete conjugation existed due to open bivalents in the PMC of the six F<sub>4</sub> hybrid plants. In four hybrid variants, available univalent chromosomes might be due to the structural differences between the homologous chromosomes caused by the exchange of separate chromosomes with alien ones.



## DISCUSSION

The narrowness of the genetic base of the existing germplasm and cultivated genotypes is one of the primary hindrances in improving cotton productivity. The use of wild species in developing promising cotton cultivars with enriched genetic diversity and considerable tolerance to biotic and abiotic stresses is one of the brilliant approaches. Developing introgression lines helps to expand the genetic diversity in cotton and increases resistance to various diseases and pests (Shavkiev *et al.*, 2022). In the presented studies, cytogenetic analysis of  $F_1$  interspecific hybrids resulted from intergenomic hybridization that revealed bivalent and univalent accessions at the metaphase-I stage of meiosis. Sanamyan *et al.* (2022) reported that in a metaphase-I analysis of 49 monosomic  $F_1$  hybrids, 47 monosomic plants exhibited 25 bivalent and one univalent modal chromosome pair.

In the latest research, three hybrid combinations, i.e.,  $F_2L-4674-77/16 \times$  Sultan,  $F_2L-95/16 \times$  Sultan, and  $F_2L-58/16 \times$  Sultan, showed the highest meiotic stability, forming 26 normally closed bivalents at the metaphase-I stage of meiosis. In the  $F_2$  interspecific combinations based on the analysis of meiosis at the metaphase-I stage in six hybrids  $F_2(G. hirsutum$  ssp. *eu-hirsutum* cultivar 'Kelajak')  $\times$  (*G. arboreum*  $\times$  *G. herbaceum*),  $F_2(G. hirsutum$  ssp. *eu-hirsutum* cultivar 'Kelajak')  $\times$  (*G. arboreum* ssp. *perenne*  $\times$  *G. arboreum* ssp. *obtusifolium* var. *indicum*), normal chromosome pairing was evident with the formation of bivalents and univalents in pollen mother cells (PMCs) (Bobokhujaev *et al.*, 2019).

The present research discovered open bivalents in PMC in five  $F_3$  interspecific hybrid plants and existing separate univalent chromosomes in two hybrid combinations. The  $F_1$ - $F_4$  hybrids showed open bivalent division in the PMC of six variants and univalent chromosomes in four hybrid variants due to the exchange of chromosomes with alien ones. It also lacked complete conjugation of chromosomes due to structural differences

between the homologous chromosomes in the hybridized forms. In similar past studies, cytogenetic analyses proceeded on interspecies hybrids, identifying the univalents, bivalents, trivalents, tetravalents, pentavalents, and hexavalents plants in cotton (Konan *et al.*, 2007, 2020; Kushanov *et al.*, 2022; Khidirov *et al.*, 2023).

In the studied cotton cultivars, meiosis metaphase-I mainly produced the bivalents with rings and rods, with univalents also forming in some genotypes. A previous study also observed bivalent, univalent, and tetravalent forms in metaphase-I of meiosis in diploid and tetraploid cotton hybrids (Noormohammadi *et al.*, 2012). The cytogenetic research on *Gossypium hirsutum* L. cultivars and their hybrids reported significant differences in chiasma frequency, distribution, and chromosome pairing, indicating their genetic differences (Sheidai, 2008).

## CONCLUSIONS

The cytological characteristics of the composite interspecific  $F_1$ - $F_4$  cotton hybrids obtained by crossbreeding of introgressive lines showed variations in the different degrees of disorders, both among the cotton lines and hybrids. Analysis of composite interspecies  $F_1$ - $F_4$  hybrids confirmed the presence of open bivalents and single univalents at the metaphase-I stage of meiosis and attenuation of synapse density. Identifying structural differences between the homologous chromosomes caused by the exchange of separate segments of chromosomes with alien ones indicates the introgression of foreign material in the studied cotton hybrids. Therefore, the recommendation to monitor the cytological stability along with morpho-agronomic traits of genetic selection materials developed through interspecific hybridization is necessary. The obtained introgressive hybrids will serve as an excellent source and opportunity for the breeders to make the genetic and breeding researches based toward introgression of useful traits of wild cotton species into genome of cultivated ones.

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