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RESEARCH ARTICLE

# Cytological and karyological analysis of interspecific cotton hybrids

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## Abstract

This article explores the morphology and karyological characteristics of cotton tetraploids, including *Gossypium tomentosum*, *G. mustelinum*, *G. hirsutum var. morili*, *G. hirsutum* L. variety AN-Bayaut-2, Bukhara-6 variety, *G. barbadense ssp. ruderale* and *G. barbadense ssp. vitifolium var. brasilense,* along with their polyploid plant generations and $F_1$ hybrids. The study describes the morphological traits and karyological features of the resulting allopolyploids. The results generated octoploid hybrid combinations, which were morphologically analyzed. It was observed that most of the octoploid plants were short, some were medium-sized and others were flowerless, displaying morphological irregularities and fragility. The leaves of these plants were thick but obtained from octoploids (C - generation exposed to colchicine). When the chromosomes of plants from $C_1$ to $C_4$ generations were counted, they returned to the tetraploid level ($2n = 4x = 52$), stabilizing the karyotype structure among the plant generations. At the same time, a process of restoration of the normal course of microsporogenesis was observed. $F_1$ hybrids obtained from these species and subspecies were observed to differ significantly in several characteristics. The first-generation hybrids exhibited distinct morphological variation. Each hybrid combination differed in traits such as speed of ripening, leaf size and shape, density, size, joint spacing in the first crop, the size of pods and overall development. The hybrid plants demonstrated robust growth, a good fruit set and notable karyological features.

## Keywords

chromosome; cotton genetics; cotton hybrid; karyotype; meiosis; microsporogenesis; mitosis; octoploid; tetraploid

## Introduction

Currently, more than 60% of Uzbekistan's commodity exports are made up of raw materials (gold, natural gas, non-ferrous metals and cotton etc.). World cotton production is growing moderately, our country produces about 3% of the world's cotton. An important trend in recent years in Uzbekistan is the rapid and continued growth of domestic cotton consumption and processing. The reduction of forecast indicators for cotton production is part of Uzbekistan's "Strategy for the development of agriculture until 2030" (1).

In the Republic of Uzbekistan, the demand for knowledge about the characteristics of chromosome structure, their sets and the mechanisms for increasing cotton productivity in the breeding program is growing. This, in turn, necessitates conducting scientific research on the similarity problems of chromosomes and creating their cytological, karyological and genetic maps. Based on these objectives, one urgent task is to improve traditional breeding methods and conduct research that will help fully utilize the global cotton gene pool (2-4).

The karyology of *Gossypium* L. species has been extensively examined in several research (5, 6). The morphology of cotton chromosomes in 10 wild and cultivated kinds, including both diploid and tetraploid species, throughout all sections of *Gossypium* L. was elucidated through these comprehensive investigations. These studies provided a foundational classification of cotton chromosomes based on their morphology, marking the first attempt to connect these characteristics. Research suggests that the inheritance of morphological characteristics in $F_1$ hybrids depends on the genetic makeup of the parent species and the geographic distance between the crosses (7).

In an interesting study, 12 novel reciprocal homozygous translocation lines were acquired in cotton (8). An important feature of these lines is the interchromosomal exchange, which was set in both heterozygous and homozygous states. This innovation identified several cotton chromosomes and their monosomes, making these lines valuable markers for cytogenetic studies.

In parallel studies, 27 varieties of *Gossypium* L. were examined and 14 types of mitotic chromosomes were analyzed, leading to an improved classification system (2, 9). As a result of the research, comprehensive information on the morphology and evolution of the chromosomes of the *Gossypium* L. species was obtained.

Despite this, the introduction of theoretical advancement in chromosome studies and conclusions into practical selection has not yet been implemented. In this regard, the present research aims to determine the impact of karyotype similarity or divergence among the parent species on the key traits such as microsporogenesis, fertility and the morphobiology of the resulting hybrids and their amphiploids. Furthermore, this study seeks to identify promising biotypes that can serve as donors of valuable traits for cotton breeding programs.

## Materials and Methods

The tetraploid species *G. barbadense* L., *G. hirsutum L., G. tomentosum, G. mustelinum, G. hirsutum var. morili, G. barbadense ssp. ruderale*, varieties AN-Bayaut-2, Bukhara-6, their $F_1$ hybrids, as well as $C_1$-$C_4$ plants (Note - The letter $C_0$-$C_4$ denotes generations obtained from experimental polyploids of cotton. C - derived from the name of the plant *Colchicum autumnale*, from which the alkaloid colchicine is obtained)., new lines and the O‘zFA-709 (created by the Institute of Genetics of the Academy of Sciences of Uzbekistan, therefore, we named it O'zFA-709.) variety from the Magnibracteolata Tod. section of cotton was studied.

The research was conducted at the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan from 2010 to 2024. The classical methods of cotton genetics and breeding and cytological and chromosome-karyological methods were used according to standard protocol (5).

Temporary chromosome study preparations were made from germinated seeds' root tips. Roots were fixed by pre-treatment in a mixture of ethyl alcohol and glacial acetic acid (3:1) for 2-3 hours. Preparations were stained with aceto-orcein (10, 11).

Chromosome morphology was investigated in the meristem cells of seedling roots at metaphase. Chromosomes were photographed at 100 x 7 magnification using a binocular laboratory microscope. Information about the microscope used Model: DN-300M with a special microphoto attachment.

Chromosome types were classified according to the conventional scheme (11). This improved the resolution of the classification system of metaphase chromosomes and simplified the boundaries between chromosome types. While the centromeric index values in standard systems are 12.5, 25.0, 37.5 and 50.0, the proposed scheme modifies them to 10.0, 20.0, 30.0 and 50.0 (Table 1).

Telocentric (T) - acrocentric chromosomes, the short arm's length is less than the chromosome thickness of a given set of chromosomes. We conditionally divided the absolute length of chromosomes into three classes:

- Class I (long) - 3.7-5.5 microns
- Class II (medium) - 1.9-3.6 microns
- Class III (short) - 0-1.8 microns

Satellite chromosomes have significant importance in the genetics and selection of cotton. Their unique characteristics and roles include:

### *Selection processes*

Satellite chromosomes allow for the rapid assessment and identification of beneficial traits in the selection process. In this context, breeders can use the genetic traits identified by satellite chromosomes to develop new varieties. For example, satellite chromosomes can be utilized to create new high-quality varieties to improve yield and disease resistance (10, 11).

### *Karyotype analysis*

Satellite chromosomes are also important in analyzing chromosome karyotypes. Changes and anomalies in the karyotype can have significant implications as they provide insights into the genetic health and stability of cotton lineages (12). The satellites of the studied species and hybrids differ according to the types of chromosomes,

**Table 1**. Details of chromosomes used to illustrate plant karyotypes

| No. | Chromosome type | Relative size (Js) |
|---|---|---|
| 1 | Metacentric (t) 50 | 40 µm |
| 2 | Submetacentric (S m) 40 | 30 µm |
| 3 | Intermediate (η) 30 | 20 µm |
| 4 | Subacrocentric (S a) 20 | 10 µm |
| 5 | Acrocentric (a) 10 | 0 µm |

which are as follows:

- The first type has a "classic" clearly defined satellite.
- The second type has a strongly coiled satellite filament and a weakly expressed constriction. The second type has a strongly coiled satellite filament and a weakly expressed constriction.
- The third type is "satellite-less," meaning no secondary constriction is observed when the satellite filament sufficiently coils.
- The fourth type has chromosomes with a partially uncoiled "diffuse" satellite (11).

Cotton chromosomes were classified by a previously published method (9). The chromosome length and thickness measurements were performed using a microscope repeated ten times. Each chromosome was described according to the following parameters:

- Absolute length (µm) - a
- Relative length - Lr
- Centromere index - Ic
- Size and shape of the satellites.

All data were analyzed using genetic-statistical methods based on ANOVA (2).

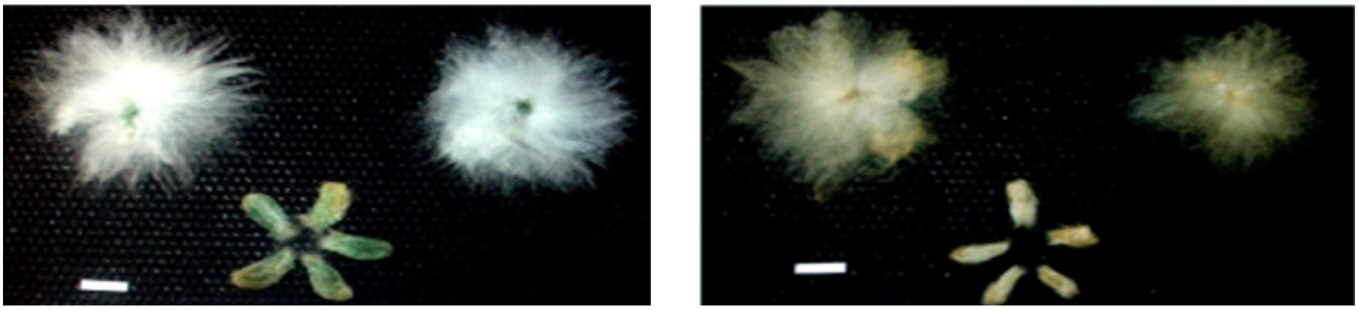
## Results and Discussion

The chromosome-karyotypic parameters for hybridization initially involved the selection process of parental species. In this process, the karyological analysis of the parental types was studied and those that are morphologically suitable are identified and separated. Hybrid seeds were then obtained, followed by the application of colchicine to induce polyploidization. This work was carried out sequentially, allowing fertile polyploids to be obtained.

The research results were conducted based on this sequence, leading to the development of octoploid hybrid combinations. Morphological analysis revealed that most octoploid plants had low heights. Some were medium-sized and occasionally bushy, exhibiting morphological unevenness, brittleness and thick leaves. The structure of the stem often had a non-traditional build and the shape and surface of the leaves were uneven. However, in some cases (for example, 8n = 104), the hybrid plants of Bukhara-6 (*G. hirsutum*) × *G. barbadense* ssp. *uderale* hybrid reached a height of up to 1.2 meters; however, their yield was low, the stems were thick, the bark was hard and brittle and the leaves were dark green with a thick leaf blade and large seed capsules, based on observation and analysis results.

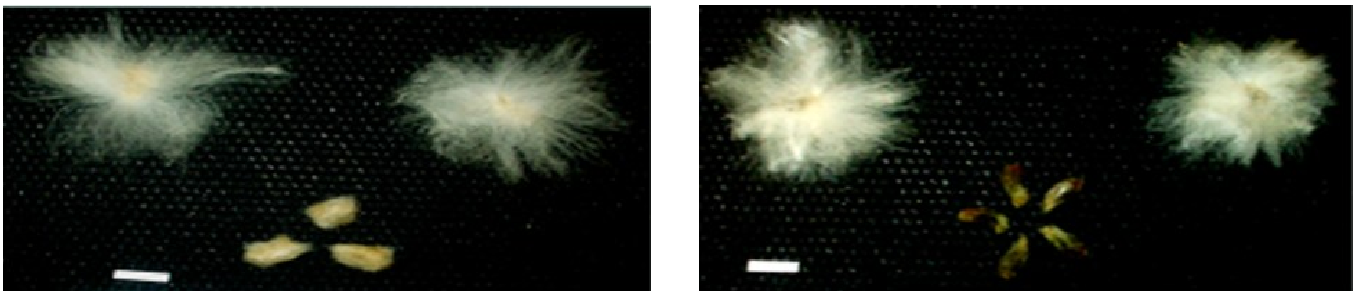
From each variant of the octaploids, 1 to 4-5 complete racemes were obtained (Fig. 1). This was achieved as a result of forecasting based on chromosome indicators (parameters) and good results were attained. Analyzing the microsporogenesis of octaploids is associated with significant challenges due to the large number of small chromosomes (8n = 104) and the scarcity of dividing maternal microspores within the microscopic field of view, which prevented us from studying the meiotic chromosome associations in the octaploids. In studying octaploid chromosomes, we limited our analysis to counting chromosomes in mitotic-somatic cells (Fig. 2).

Plants derived from octaploids (S-generation, affected by colchicine) were analyzed up to the $C_1$–$C_4$ generations. Chromosome counts returned to the tetraploid level (2n = 4x = 52), indicating stabilization of the karyotype structure across generations. Concurrently, the normal course of microsporogenesis was restored. In the morphological description of the $C_3$ and $C_4$ hybrids obtained from octaploid plants, $C_3$ *G. hirsutum* var. *morili* x *G. tomentosum* plants were found to be medium-height, reaching up to 80.4 cm, with 17.2 branching spikes; the shape of the stem was pyramidal, with branching type I-II, being unlimited. The stem was moderately pubescent, with strong antixenosis and the



**A)** $C_o$ *G. Hirsutum* Lx *G. barbadense ssp brasilensee.*

**B)** $C_o$ *G. Hirsurum* L *ssp euhirsutum* x *G. mustelinum.*



**C)** $C_o$ *G. tomentosum* x *G. mustelinum.*

**D)** $C_o$ *G. Hirsutum* L x Co *G. hirsutum* L var. mori.

**Fig. 1**. Seed and fiber of octaploids.

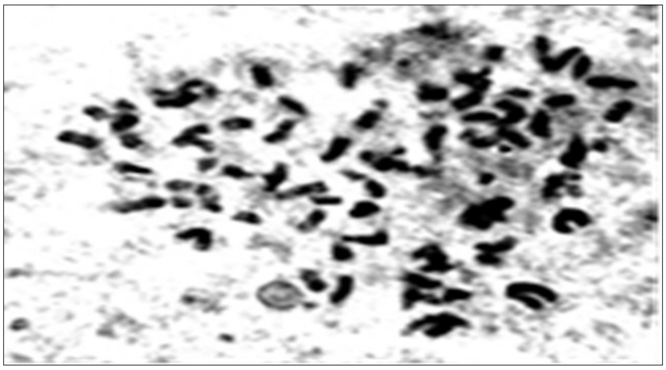


**Fig. 2.** C$_1$ *G. hirsutum* variety Bukhara-6 x *G. barbadense* ssp. ruderale - metaphase plate.

height of the first spike emergence, hs (the number of nodes up to the first fruiting branch of the cotton plant), was 6.2. The leaves were medium-sized, dark green and palmate. The shape of the raceme was egg-like. The seeds were hairy. The number of locules in the raceme was 4-5. To gain insight into hybridization, experimental polyploidy and the chromosomal changes that arise in the stabilization process of karyotypes in subsequent generations, we first present information on the karyotypes of the parent and descendant species. The primary sources used in this study included *G. tomentosum*, *G. mustelinum* and *G. hirsutum var. morili,* among others. These cotton species belong to the *Gossypium* L. *Magnibracteolata Tod.* section and share a chromosome set of 2n=4x=52. The karyotype of the AN-Bayaut-2 variety (2n = 4x = 52) includes 12 pairs of metacentric, 13 pairs of submetacentric and 1 pair of acrocentric chromosomes. The second pair consist of long acrocentric chromosomes, 6 pairs of long submetacentric, 10 pairs of medium submetacentric chromosomes and 20 pairs of short submetacentric chromosomes, with only 4 pairs of satellite chromosomes. The total length of all chromosomes in the diploid set of the AN-Bayaut-2 cotton variety equals 138.84 + 2.5 micrometers. The average length of a single chromosome amounts to 2.67 micrometers (Fig. 3).

We briefly analyzed some characteristic structural features of the karyotypes of interspecific F$_1$ hybrids. From Fig. 4 (a, b), it is evident that the arrangement of chromosomes in the karyotype of F$_1$ *G. mustelinum* x *G. tomentosum* is not "uniform" in ladder lengths, similar to the pure species shown in Fig. 3. The stepwise disruption of the chromosomes, i.e., the idiogram structure, is characteristic of this karyotype. All other F$_1$ hybrids in our study also did not exhibit uniform step gradation in length. This form of the idiogram and its peaks (contours) indicated that these hybrid chromosomes are grouped according to several morphometric parameters resembling their morphometric characteristics. Such a representation of chromosome arrangement in the idiogram may likely lead to disturbances in conjugal formation during meiosis, including disturbances in the meiotic

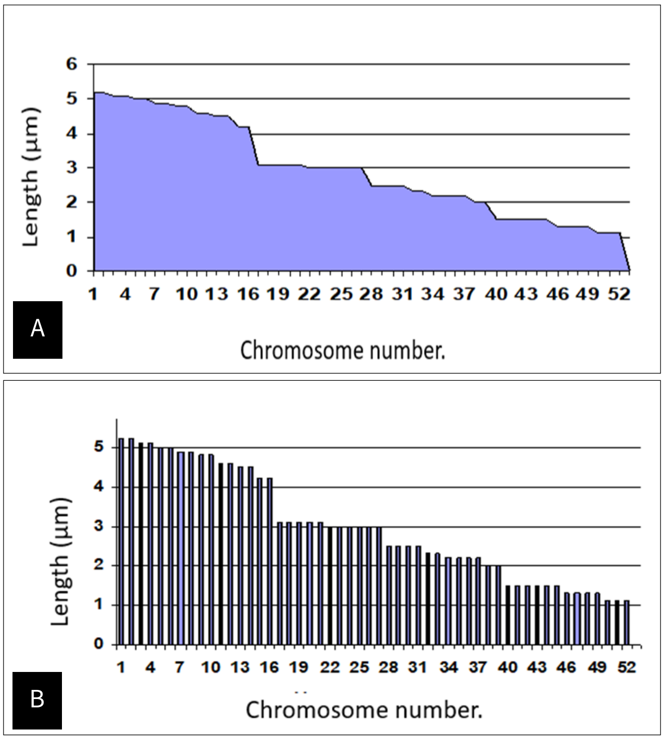


**Fig. 4. A-** F $_1$ *G. mustelinum* x *G.* chromosome idiogram, **B-** Chromosome idiogram location points (contour).

Thus, the analysis of the structure of interspecific F$_1$ hybrid karyotypes involving species belonging to the Magnibracteolata Tod. section indicates that all F$_1$ hybrids possess a characteristic image of their karyotype chromosome idiogram, including unique position points and contours. Furthermore, it demonstrates that the length distribution of chromosomes in the idiogram is not uniform, lacking the straight and even characteristics typical of the species and their descendant species (Fig. 5). It has been established that there are similar groups in the morphology of chromosomes in F$_1$ plants belonging to the Magnibracteolata Tod. section. Each F$_1$ plant has a specific number of similar chromosomes, corresponding to unique quantitative characteristics specific to those plants.

Therefore, we believe that the karyotypes of the first-generation interspecific hybrids are not stable and they begin to stabilize starting from the F$_2$ generation. Karyotype analyses of the F$_1$ hybrids indicate that positive results were

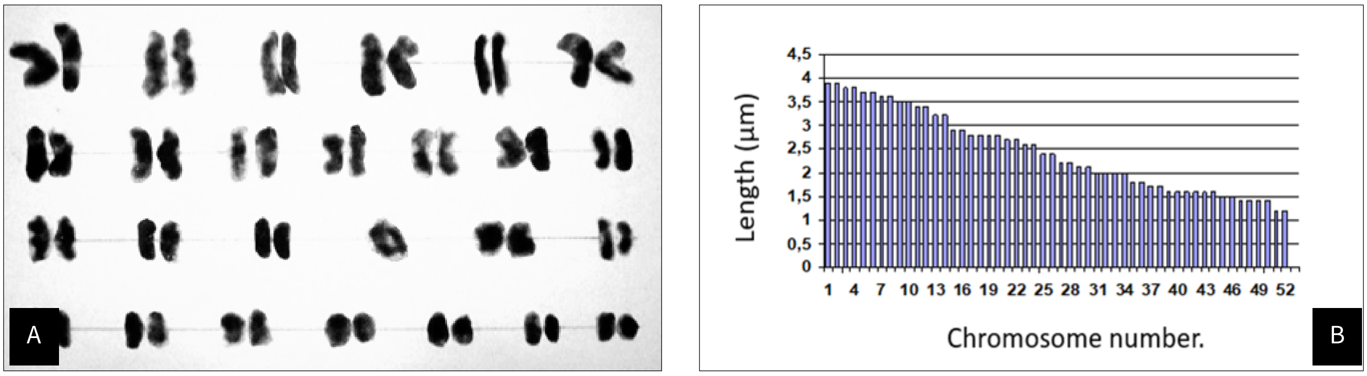


**Fig. 3. A-** Chromosome idiogram of AN-Bayaut-2 variety. **B -** Scheme of chromosome idiogram (*G. hirsutum* L.) (2n=4x=52).

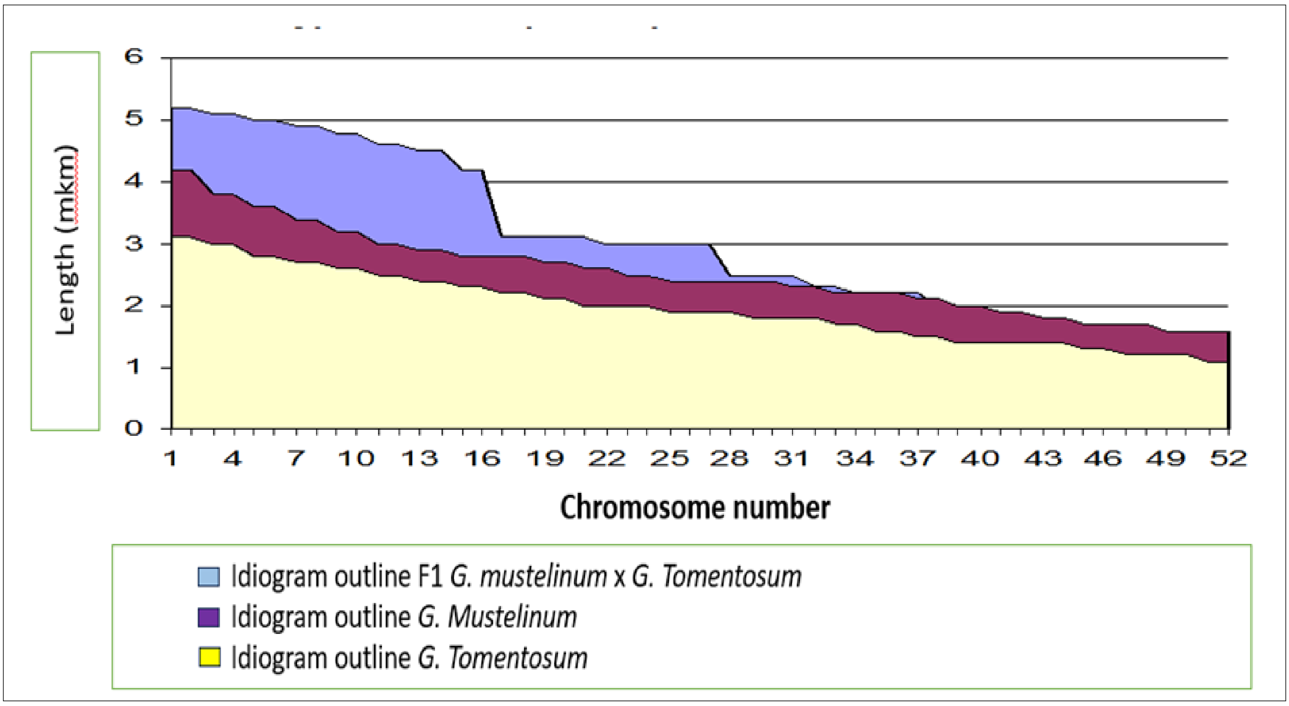


**Fig. 5.** $F_1$ *G. mustelinum* x *G. tomentosum* comparison of the karyogram of the hybrid (chromosome idiogram location points) with the karyograms of the original species.

achieved through a twofold increase in the number of chromosomes (allopolyploidy) in all $F_1$ hybrids.

In $F_1$ hybrids, the meiotic process often proceeds normally. The high quantity of mature pollen grains in the $F_1$ hybrids suggests that minor errors in the meiotic process do not affect the complete development of the pollen.

The initial species, interspecific $F_1$ hybrids, allopolyploids and plants obtained from allopolyploids were described based on analyses conducted at the end of each growing season. These $F_1$ hybrids, obtained from parent and descendant species, differ in many characteristics. The first-generation hybrids are morphologically diverse. They vary in traits such as growth rate, leaf size and shape, density, size, internode length of the first fruiting spike and size of the racemes, with each hybrid combination differing from one another. Hybrid plants develop well and produce fruit.

Furthermore, the cultivation of $F_1$ hybrid plants was excluded when using the method improved by us. The results and analyses of the conducted research fully align with the opinions of researchers who have confirmed that the octaploid (8n = 104) chromosome set represents a limited polyploidy level for the *Gossypium* L. genus.

## Conclusion

As a result of the conducted research, it was concluded that with chromosomal-cytological data in cotton, it is possible to select pairings for hybridization, leading to the emergence of new forms of practical and scientific interest. It was determined that after octaploids (8n = 104) revert to tetraploid level (4n = 52), there are fewer errors in microsporogenesis in hybrids such as *G. hirsutum*x *G. tomentosum*, *G. mustelinum* x *G. tomentosum*, *G. barbadense* x *G. tomentosum* and others. Based on the chromosomal-cytological data of cotton, it was concluded that the new forms obtained can be used as donor and initial materials in selection work.

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## Authors' contributions

MM was the lead specialist in cytogenetic research and a key participant in writing the main part of the article and conducting statistical analysis. KM, SY, DI and AL participated in laboratory analysis and prepared reports based on the experiments conducted. MZ, MD, KS and ZN carried out field experiments and statistical analysis. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used generative AI to assist in translating biological and genetic terms and processes. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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