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Cytological analysis of interspecific hybrids of cotton

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Abstract

The article analyzes data on the production of new intergenomic hybrids involving the species *Gossypium thurberi* Tod., *Gossypium raimondii* Ulbr., *Gossypium arboreum* L., *Gossypium hirsutum* L., *Gossypium barbadense* L., belonging to genome groups D1, D5 A2 cotton and presents the results of some cytological studies of the resulting hybrids. As a result of experiments using the method of interspecific hybridization, an amphidiploid {[(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × *G. hirsutum* L.} with a complex genetic basis was obtained. Based on the hybridization of the resulting amphidiploid with cultivars of the species *G. barbadense* L. and *G. hirsutum* L., interspecific complex hybrids were synthesized, including 4 species with hybridization of 46.7-55.1% and including 5 species with hybridization of 24.6-31.3%. By interspecific hybridization and backcrosses, valuable source material for the cotton breeding has been created. Valuable introgressed lines having high productivity and high quality, which are at various stages of testing

have been realized. Cytological analysis of the hybrids revealed that they were true interspecific crosses. Observations of meiotic metaphase chromosomes indicated the degree of relatedness between species.

Introduction

Today, the global transformation of the environmental balance on a global scale has also affected the cotton sector which plays an important economic role. Cotton is the most important natural fiber in the textile industry, as well as a vital agricultural product in the world economy. The annual value of the global cotton harvest is estimated at over 30 billion dollars, with plush fibers accounting for 90% of this value.[1,2] Cotton culture and processing employs 350 million people worldwide. The economic importance of cotton for the global textile industry has sparked great interest in improving the genetic potential inherent in this culture in the selection of cultivars. The main focus on cotton growing is to create new varieties which are naturally resistant to various stress factors, and which are fertile and have high fiber quality.[3] This assumes the widespread use of the potential of wild and semi-wild species in the cotton collection in breeding and genetic processes when creating new varieties.[4,5]

The rational and effective use of the potential of the existing wild and cultivated species of cotton, involving them in interspecies hybridization, obtaining genetically enriched unique hybrids of cotton, allows the creation of new varieties that are superior to the currently cultivated varieties in terms of main economic characteristics. Therefore, it is important to create new multi-genome interspecies complex hybrids based on the hybridization of existing amphidiploids of cotton with different genome structure, to carry out research on their use in selection-genetic studies and practical selection processes.

On the basis of the analysis of literary sources, it has been proved that by using the method of cross-species hybridization of cotton plant it is possible to achieve wide variation in terms of valuable economic characters, as well as to create opportunities to carry out selection work to obtain the desired economic character.[6] Enrichment of the *Gossypium hirsutum* genome with alleles of economically valuable genes from other cotton species is very important.[7]

Wild *Gossypium* species (Malvaceae) possess massive amount of unexplored genetic diversity that can be exploited to broaden the genetic base of cotton.[8]

Currently, new and important interspecific lines, families and varieties of cotton have been identified in interspecific 3-4-5 species hybridization studies (*G. hirsutum* L., *G. barbadense* L., *G. thurberi* Tod., *G. raimondii* Ulbr. and *G. arboreum* L.) ; they can serve as starting material in practical selection of cotton and genetic selection research.[9] However, it is known that wild and

semi-wild forms and species belonging to different genomes of cotton are difficult to mix with cultivated varieties, and the fact that most of the hybrid generations obtained with them show signs of impotence and infertility has led to their infrequent use in practical selection.[10,11] Therefore, great attention has been paid to the study of the causes of difficult cross-breeding, infertility and infertility symptoms in the hybridization of cotton between 2-3 genome species.[5] It is known that wild and semi-wild forms and types of cotton belonging to different genomes are difficult to cross with cultivated varieties, and the fact that most of the interspecies hybrids obtained with them show symptoms of infertility has led to their infrequent use in practical breeding.[12]

However, the study of the development of the hybrid sex circle between *G. hirsutum* L. (C-4727 variety) × *G. trilobum* species revealed the presence of a large number of disorders in the course of meiosis, micro, macrosporogenesis and gametophyte development.[13-15] This has been shown to result in significant pollen and seed shoot sterility.

Cotton's development and progress have benefited greatly from interspecific hybridization. Cotton is unique among crop plants in that four distinct species of the genus *Gossypium* were domesticated for lint fiber production on two separate continents.[16,17]

Given their complementary economically valuable traits, numerous attempts have been made to hybridize the two species*, G. hirsutum* L., *G. barbadense* L., through traditional breeding.[18] A research in this regard was carried out in a very broad sense in the middle of the last century within the framework of the study of the sexual development of hybrids between cultivated and wild, diploid and tetraploid species which means that the presence of a large number of defects in the development of micro-, macro-sporogenesis and gametophytes in the process of meiosis was determined, and a significant number of pollen and seed shown to cause shoot sterility.[19] The scientists of our republic skillfully used the method of polyploidization in order to carry out free hybridization of wild diploid species with cultivated varieties.[20,21] As a result, interspecies (3-4 species) hybrids have been created with the participation of 2, 3 and 4 species of cotton with different genome structure, in other words cultivated and wild, semi-wild species.[22,23] On the basis of the study of their descendants, a number of initial materials with genetically enriched rare traits and characteristics have been created and are being effectively used in practical genetics and selection processes.[11] Rare amphidiploids that cross easily with cultivated tetraploid varieties of cotton have been obtained, and by crossing them with tetraploid species belonging to cultivated (AD)1 and (AD)2 genomes, a completely new amphidiploid with 4 species has been synthesized. Compared to other polygenome hybrids, this amphidiploid is easy in terms of crossbreeding, it has high economic and fiber quality characteristics, and resistance to various diseases and stress factors.[24] Moreover, other research workers have attained success in introgression of *Gossypium hirsutum* L. and *Gossypium arboreum* L.[25]

The aim of this study was to reveal the possibilities of using the interspecific hybridization of the species *G. hirsutum* L. with some wild diploid species in the cotton breeding in our country to improve productivity, fiber quality and resistance to some stress factors of the modern varieties.

Materials and Methods

The methodology for obtaining new intergenomic cotton hybrids with a complex structure having 4 and 5 species and the results of some cytological studies of the resulting hybrids were analyzed.

An amphidiploid cotton hybrid belonging to the genomic groups D1, D5 A2 F_1 (F_1 F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L. (conventionally numbered as K-28), varieties C-6524, C-4727 and Omad belonging to the AD1 genome *(G. hirsutum* L.), and variety Termiz 31 belonging to the AD2 genome (*G. barbadense* L.) was studied.

Cytological studies

Chromosome number and morphology have been measured at the metaphase stage of the meristem of the newly growing seedling. For this purpose, cut-off seeds have been placed in a Petri dish at a temperature of 25-26ºC, 0.8-1.2 cm roots have been put in a saturated aqueous solution of paradichlorobenzene for 2-3 hours, and then frozen overnight in Carnois fixative. The specimens for microscopic observation have been prepared in two different ways. In the first method, the rhizomes have been left in a paint consisting of acetorocein and lactic acid (in the ratio of 10:1) (for two days and nights) and temporary preparations have been prepared and examined.[19]

In the second method, the rhizomes frozen in the Carnois fixative were stained with fuchsin sulfic acid (Schiff's reagent) and observations were made. Young developing buds were collected in the field and fixed in Carnoy's fluid 60% ethanol, 30% chloroform and 10% glacial acetic acid, 1 gram of ferric chloride in quick succession between 9.30 and 10.15 am on bright sunny days.[26] Traces of iron as ferric chloride was added to the bottle of acetic acid and kept after filtration to be used in the fixative for improving the stainability of the chromosome. After keeping the fixed materials under low temperature (16°C) for a minimum period of 4 h, the buds were washed in tap water and stored in 70% ethanol. Three or four anthers were squashed on a slide with a drop of 1% acetocarmine and examined under a microscope.

To determine the morphology of the chromosomes of hybrid samples and their parental forms, we used a new technique presented by Muratov and Sadikov which gives results accurate to a hundredth of micrometers.[27] We have observed the process of meiosis in the mother cells of the studied samples by freezing the bolls in Newcomer's fixator and staining them with ferric acetic carmine. Various changes, integrations and characteristics of chromosomes during the metaphase I (MI), metaphase II (MII), anaphase I (AI) and anaphase II (AII) stages of the meiosis process have been identified and

analyzed. In addition, by staining pollen grains with acetocarmine, pigmented and non-pigmented pollen grains, as well as the tetrad stages of microspores have also been studied. All cytological observations were carried out using microscopes AxioScopeA1, Laboval (Carl Zeiss, Aalen Germany) and Biomed (Leica, Widnau Switzerland) with a 10× to 100× increase in lenses binocular nozzle of 1.6× and PeriPlan GF 12.5× and a 10× eyepiece. Microphotography was performed using a Zeiss AxioCam ERc 5s digital camera (Carl Zeiss, Aalen Germany).

Results

The species involved in the complex interspecific hybrid of cotton [F_1*(G. thurberi* Tod. × *G. raimondii* Ulbr) × *G. arboreum* L.] × *G. hirsutum* L. belonging to D1, D5, A2, AD1 genomes have been identified in research and have been called intergenome complex hybrids involving 4 species. The complex intergenomic hybrid {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr) × *G. arboreum* L.] × *G. hirsutum* L.} × *G. barbadense* L. belonging to D1, D5, A2, AD1, AD2 genomes, have been called hybrids involving 5 species. The details on materials used in the study are given in Table 1.

Interspecific hybridization in cotton

To obtain a complex intergenomic cotton hybrid, we used four species: *G. thurberi* Tod, *G. raimondii* Ulbr, *G. arboreum* L., and *G. hirsutum* L. distinct into two varieties S-6524/S-4727. Species were taken as the maternal form, i.e.: a) {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S -6524} and b) {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727}. For *G. hirsutum* L. two different varieties were used which are the fourth species. That is, in the first crossing, the S-6524 variety was used, and in the second hybrid, S-4727 was used, both belonging to the *G. hirsutum* L type. Since the initial plants obtained in the mother form have photoperiodism, photoperiodic conditions were created for their hybrids using black-and-white films. As a result, new 4^{th} and 5^{th} interspecies hybrid plants of yielding cotton have been obtained.

In order to determine the level of crossbreeding in the process of obtaining a new complex intergenomic hybrid of cotton, we have paid attention to the number of outcrossed flowers and the number of pods born normally (Table 2). According to the table 2 data, four new different complex intergenomic hybrids of cotton were obtained. The number of flowers crossed between {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} and Omad variety was 62, of which 29 normally born bolls have been obtained. In the 4-genome hybrids with S-4727 variety as the mother, 32 normally born cysts were obtained when 58 Omad varieties have been crossed. If we convert these calculations into percentages, the volatility in the first combination is 46.7%, and in the second combination it is 55.1%.

The number of flowers crossed between the hybrid {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} and the Termiz 31 variety was 73; when obtaining a complex intergenomic hybrid involving 5 types of cotton only 18 of them had normal scores. In 4 different hybrids with S-4727 as the maternal form, 21 normally born cysts were obtained when 67 units of Termiz 31 were crossed. If we convert these calculations into percentages, the volatility in the first combination is 24.6%, and in the second combination it is 31.3%. If we compare the quantitative indicators of the level of crossbreeding in the process of obtaining complex intergenomic hybrids with the participation of the 4th and 5th species, it can be found that in the second option the indicators for obtaining complex intergenomic hybrids with the participation of 5th species are 2.5-3.0 times less than the hybrids of the first option.

In the course of research, the degree of seed filling and immaturity has been studied on the basis of such characteristics as the appearance, weight, laboratory germination capacity, and germination of seeds in pods obtained from complex intergenomic hybrids.[24] According to the table 2, in obtaining the complex intergenomic hybrid with the participation of 4 new types of cotton {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} hybrid and the Omad variety, the total 76.5% of seeds, {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} × Omad hybrids and 78.7% of completed seeds have been separated.

However, it was noticed that the number of mature seeds in seeds obtained from capsules of complex intergenomic hybrids involving 5 new species decreased sharply. In particular, the number of immature seeds compared to the total number of seeds in hybrids obtained from crossing the hybrid {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} with the Termiz 31 variety amounted to 72.5%, and in hybrids obtained from crossing {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} × Termiz 31 19.4 % mature seeds were selected.

Cytological confirmation: meiosis in parents

The importance of cytological research in plant cells is incomparable in eliminating the defects and deficiencies manifested in complex interspecies hybridization, in preventing various incompatibilities in the normal development of plant cells, and in determining the causes of withdrawal.[28] The variation of the chromosome number and morphological characteristics in the karyotype of the synthesized complex intergenomic hybrids were analyzed in comparison with those of the parental forms. Figure 1 shows some photomicrographs of chromosome groups in somatic cell karyotypes of plants belonging to the studied complex intergenomic hybrids and parental forms. First of all, on the basis of microphotographs, the variability of the number of chromosomes and the amplitude of the oscillation in the karyotype of all studied samples, parental forms and complex intergenomic hybrid plants have been studied. According to the obtained results, even though all the studied samples are tetraploid

plants, it was found that the oscillation amplitude of the number of chromosomes in their somatic cells is different. If we focus on the analysis of the average number of chromosomes in the karyotype of the hybrids, {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} and {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} the average number of chromosomes of the complex intergenomic hybrid is 52.02 ± 0.05 and 52.09 ± 0.05, respectively; the oscillation amplitude is 48-56 organized small classes. The number of chromosomes of Omad (*G. hirsutum* L.) and Termiz 31 (*G. barbadense* L.) varieties obtained in the parent form is 52.04 ± 0.02 and 52.07 ± 0.03 respectively. The difference in the number of chromosomes of the varieties obtained in the parent form from the hybrids in the mother form is mainly in the amplitude of the oscillation of the number of chromosomes in the hybrids. It means that the amplitude of variation of the number of chromosomes of the parent varieties is in the range of 48-56 classes, while in the hybrids of the maternal form it comprises the 50-54 classes. A sharp change in the number of chromosomes and a difference in the amplitude of their fluctuations in sharp changes compared with those of the parents were determined, especially hybrids with 5 species {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S -6524} × Termiz 31 and {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} × Termiz 31 were more significant compared to the parents.

The number of chromosomes in the 1st type hybrids of cotton is 51.85 ± 0.05 and in the 2nd variant hybrids, the number of chromosomes is 51.12 ± 0.04 The amplitude of fluctuations in the number of chromosomes of both hybrids is in the range of 48-56 and it has been established that samples of the parental form in terms of class are much wider in the amplitude of fluctuations. This can have a negative effect on the hybridization process which is on the conjugation of chromosomes. Because to meet chromosomes in a normal conjugation, the chromosomes of the father and mother plants try to find their homologues, *i.e.* their pairs, as much as possible. Based on the results, it was also found that in the hybrids obtained in the mother form, even in the parent plants with a constant number of chromosomes, there are aneuploid cells. Chromosome number variability which is oscillation amplitude, is also evident in F_1 and F_2 plants of newly obtained interspecific hybrids.

The identification and analysis of morphological characters of chromosomes of plants, including cotton varieties and hybrid plants, can be the next step after counting them. We have used a rapid method with an accuracy of 0.01 μm to measure the morphological indicators of chromosomes. Based on the positive and effective use of this method, indicators such as the length, thickness of chromosomes, and the total length of chromosomes in the karyotype of the studied samples were comparatively studied with high accuracy in order to determine the homologous similarity of chromosomes. The average length of complex hybrid chromosomes (one chromosome) {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} obtained in the mother form was 2.08 ± 0.03 μm, and the total karyotype length of chromosomes was 108.34 ± 0.03 μm. It has been

found that the average length of chromosomes, the length of the total chromosomes in the karyotype, and even their thicknesses were significantly different in Omad and Termiz 31 varieties obtained in the paternal form. A sharp difference in the morphological characteristics of the chromosomes of the father and mother forms of hybrids will, of course, negatively affect the normal conjugation of chromosomes during hybridization, and the micro- and macro-sporogenesis that takes place during meiosis, that is, the hybridization process. As evidence of this, it is possible to cite the degree of crossbreeding during the acquisition of complex intergenome hybrids with the participation of species 4 and 5 mentioned above (Table 3). In the karyotype of the hybrids obtained with the complex hybrid {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} with the participation of Omad and Termiz 31 varieties, the morphological indicators of chromosomes are characteristically seen.

In particular, it has been found that the morphological indicators of complex intergenomic hybrid chromosomes with the participation of 4 species are mainly located between parental form indicators. The thickness of the chromosomes is almost constant and does not differ sharply from that of the parental forms. In the experiments, the hybridization rate is the highest (46.7%) in obtaining complex intergenome hybrids with the participation of 4 species. As for the length of chromosomes, the average length is 2.15 ± 0.04 μm, which is higher than that of the mother form, and slightly lower than that of the father form.

In the karyotype of the hybrids obtained with the participation of the Termiz 31 variety, the morphological parameters of the chromosomes are characteristically changed, compared to the hybrid with the mother form and the previous 4 species or the hybrid obtained in the first combination; all parameters of the chromosomes (length, thickness, vibration amplitudes according to these parameters, etc.) have been found to be much lower. Therefore, in the experiments, hybrids with participation of 5 species have the lowest rate of crossbreeding (24.6%). The analysis of research results showed that for a normal conjugation in the process of meiosis of interspecific complex hybridization of plant chromosomes participating as paternal and maternal forms, the length and thickness of the chromosome is of great importance when finding their homologs. Therefore, only 24.6% hybridization has been achieved in obtaining complex intergenome hybrids involving 5 species. The results of the new research obtained in this regard are important for cotton genetic selection, especially for the practical selection of cotton.[29]

Figure 2 shows typical photomicrographs of normal and abnormal tetrads observed in pollen grains of complex intergenomic hybrids. The figure shows {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} and {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} cotton sample photomicrographs of pink (large black grains) and non-pink (small, light black

grains) pollen grains of the flowers of new hybrid plants with 5 species obtained by crossing Termiz 31 with a complex intergenomic hybrid given.

Discussion

According to the analysis of data presented in the literature, the degree of germination of hybrids obtained with the participation of new and old cotton species has decreased, the number of full-fledged seeds in seeds obtained from pods has decreased, disturbances in metaphase 1 of the meiosis process have occurred disturbances in metaphase 1 of the meiosis process and, as a result, di-, tri- and polyads instead of tetrads occurredand this leads to the fact that the grains become sterile.[7]

The main reasons for the sharp difference in the level of hybridization in complex intergenomic hybrids with the participation of the 4th and 5th species and the observed sharp differences between the completeness of their seeds are due, firstly, to the difference in the period of hybridization and secondly, to the growing conditions of 10-15 days, and the chromosome number and morphology of the parental plants. Thirdly, it is possible to point out different deviations that occur in the process of microsporogenesis during the crossbreeding of the flowers of the parent plant.

In cytological studies, we focused on the comparative analysis of the new 4th and 5th different complex intergenomic hybrids of cotton and their parental forms in the pollen grains spores of S-6524 and S-4727 varieties. When obtaining interspecies hybrids, due to the fact that pollen grains are not compatible with genetic and functional characteristics, the appearance of various defects observed in the process of meiosis is determined by the percentage of tetrads in the meiosis, and it is also referred to as “meiotic index”. Many breeders also associate this indicator with plant productivity. However, the number of tetrads alone is not sufficient for such an assessment and for explaining the occurrence of various defects which were observed in the process of meiosis. Therefore, in the analysis of the meiosis process of plants, monad, dyad, triad and polyad were taken into account, as well as the tetrads in the stem, and the percentage of anomalous tetrads was analyzed.

According to the obtained results, the amount of normal tetrads of S-6524 and S-4727 varieties of cotton, which took part in the hybridization in the form of a father, has the highest index and made up 95.4 and 96.8% respectively (Table 4). In interspecies hybridization, this index is 90.5% and 92.7%, respectively, in complex intergenome hybrids of 4 different maternal forms involving varieties S-6524 and S-4727. It has been found that the number of normal tetrads observed in the newly obtained complex intergenomic hybrid plants is much lower than that of the father and mother forms. In particular, in the 5th *G. barbadense* L. variety obtained with the participation of the Termiz 31 variety {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} × Termiz 31 and {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} × Termiz 31, the number of normal tetrads in complex obtained intergenomic hybrids is 76.3 and 78.0%, respectively. These indicators

show a difference of 15.0 - 20.0% compared to paternal and maternal forms; 8 monads, 15 dyads, 21 triads, 196 tetrads, and 17 polyads have been found in 257 spores studied in the first combination, and 7 monads, 13 dyads, 11 triads, 177 tetrads, and 19 polyads were found in 227 spores studied in the second combination. The anomalies manifested in the pollen spores of these hybrids have a negative effect on plant pollen and pollen grains, resulting in poor pollination of flowers. Furthermore, in these hybrids the number of defects observed in the process of meiosis caused an increase in the number of anomalous tetrads along with normal tetrads due to the failure of normal chromosome conjugations. Cytogenetic studies were carried out on *G. thurberi* Tod. × *G. raimondii* Ulbr. belonging to genome group D and in interspecific hybrids belonging to different genomes *G. arboreum* L. × *G.thurberi* Tod, *G. herbaceum* L. × *G.thurberi* Tod. When analyzing hybrids, linking chromosome conjugation during meiosis with fertility of hybrids of *G. thurberi* Tod. × *G.raimondii* Ulbr. are the most fertile and their chromosomes form normal bivalents. The amounts of anomalous tetrads in studied hybrids {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-6524} × Termiz 31 and {[F1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × S-4727} × Termiz 31 combinations are 23.7% and 22.0%, respectively. It means that while obtaining these hybrids, it has been found that the level of hybridization is very low and the amount of incomplete seeds was also high. Nevertheless, it has been found that the plants obtained from this hybrid are most productive, and fiber quality and many valuable traits are much higher compared to the paternal and maternal forms. It is discernible from the present study that the greater homology observed between A and D genomes aids in production of desirable recombinants despite minor cytological disturbances as there are successful boll setting and viable seed production. The development of stable intergenomic hybrids requires in-depth knowledge of the chromosomal behavior of the hybrids. Cytological analysis of the hybrids revealed that they were true interspecific crosses. Observations of meiotic metaphase chromosomes indicated the degree of relatedness between species.

Conclusions

As a result of experiments using the method of interspecific hybridization, an amphidiploid {[(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × *G. hirsutum* L.} with a complex genetic basis was obtained. Based on the hybridization of the resulting amphidiploid with cultivars of the species *G. barbadense* L. and *G. hirsutum* L., interspecific complex hybrids were synthesized, including 4 species with hybridization of 46.7-55.1% and including 5 species with hybridization of 24.6-31.3%. The difference in the degree of crossing is based on the dependence of the 4^{th} and 5^{th} species involved in hybridization. It was determined that chromosomes are characterized by normal conjugation and the formation of bivalents in cells; the nature of disorders and defects at the metaphase (MII) and anaphase (AII) stages of meiosis affects the meiotic index, resulting in a sharp

difference depending on the production of the intergenomic hybrids complex with the participation of 4^{th} and 5^{th} species.

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Table 1. Scheme of obtaining new intergenomic hybrids of cotton with 4 and 5 species.

1. Wild forms and cultivars of different genomes of cotton involved in obtaining complex intergenome hybrids	*G. thurberi* Tod. (D_1 genome); *G. raimondii* Ulbr.(D_5 genome); *G. arboreum* L. (A_2 genome); *G. hirsutum* L.$(AD)_1$ genome and *G. barbadense* L. $(AD)_2$ genome.
2. different complex hybridization schemes maternally involved in obtaining complex intergenome hybrids	♀ F_0[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × *G. hirsutum* L. A) [F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.) × C-6524]; B[F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.) × C-4727].
3. Cultivated varieties involved in the paternity in obtaining complex intergenome hybrid	♂Omad (*G. hirsutum* L.) and ♂ Termiz 31 (*G. barbadense* L.)
4. Complex intergenomic hybridizations involving 4 species of cotton:	a) BC_1 [F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.) × C-6524] × Omad; b) BC_1 [F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.) × C-4727] × Omad; c) BC_2{[F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.) × C-6524] × Omad } × Omad; d) BC_2{[F_1(F_1 *G. thurberi* Tod. × *G.raimondii* Ulbr.) × *G. arboreum* L.) × C-4727] × Omad } × Omad.

5. Complex intergenomic hybridizations involving 5 species of cotton:	a) BC_1 [F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr) × *G. arboreum* L.) × C-6524] × Termiz 31; b) BC_1 [F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr) × *G. arboreum* L.) × C-4727] × Termiz 31; c) BC_2{[F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr) × *G.arboreum* L.) × C-6524] × Termiz 31} ×Termiz 31; d) BC_2{[F_1(F_1 *G. thurberi* Tod. × *G. raimondii* Ulbr) × *G. arboreum* L.) × C-4727] × Termiz 31} × Termiz 31.

Table 2. Interbreeding rate and number of completed seeds in obtaining new complex intergenomic hybrids of cotton involving 4 and 5 species

Complex hybridization scheme	Crossed number of flowers	Number of tied bo Pcs	Crossing rate, %	Completed seeds, %	Incomplete seeds, %
Pattern of hybridizations to obtain the new 4 different complex intergenomic hybrids of cotton: {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × *G. hirsutum* L.} × *G. hirsutum* L.					
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524}× Omad	62	29	46.7	76.5	23.5
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-4727} × Omad	58	32	55.1	78.7	21.3
Pattern of hybridizations to obtain the new 5 different complex intergenomic hybrids of Cotton: {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × *G. hirsutum* L.} × *G. barbadense* L.					
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Termiz 31	73	18	24.6	27.5	72.5
{[{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-4727} } × Termiz 31	67	21	31.3	19.4	80.6

Table 3. Some morphological features of chromosomes in somatic cell karyotypes of complex intergenomic hybrid cotton plants.

Parent forms and hybrid combinations	Average length of chromosomes, µm	Average thickness of chromosomes, µm	General karyotype brain chromosomes length, µm
♀ {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524}	2.08±0.03	0.88±0.07	108.34±0.03
♂ Omad (*G. hirsutum* L.)	2.17±0.03	0.78±0.02	115.13±0.03
♂ Termiz 31 (*G.barbadense* L.)	2.03±0.05	0.61±0.03	105.70±0.05
{[F_1(*G. thurberi* Tod. × *G.raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Omad	2.15±0.04	0.83±0.05	111.84±0.03
{[F_1(*G. thurberi* Tod. × *G.raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Termiz 31	2.10±0.07	0.77±0.05	108.88±0.06

Table 4. F1 plants of new 4 and 5 species of cotton and normal tetrads in pollen grains of parental forms and anomalous spore counts. pcs: pieces.

Parent forms and hybrid combinations	Number of cells studied	Monads, pcs	Dyads, pcs	Triads, pcs	Tetrads, pcs	Polyades, pcs	Abnormal spores, %	Normal tetrads, %
♀ {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524}	250	3	8	6	226	7	9.5	90.5
♀ {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-4727}	275	2	4	5	255	9	7.3	92.7
♂ Omad (*G. hirsutum* L.)	130	-	1	3	124	2	4.6	95.4
♂ Termiz 31 (*G.barbadense* L.)	154	-	2	2	149	1	3.2	96.8
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Omad	265	5	7	9	229	15	13.6	86.4
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Termiz 31	257	8	15	21	196	17	23.7	76.3
{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-4727} × Omad	243	3	9	8	212	11	12.8	87.2

{[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-4727} × Termiz 31	227	7	3	1	177	19	22.0	78.0

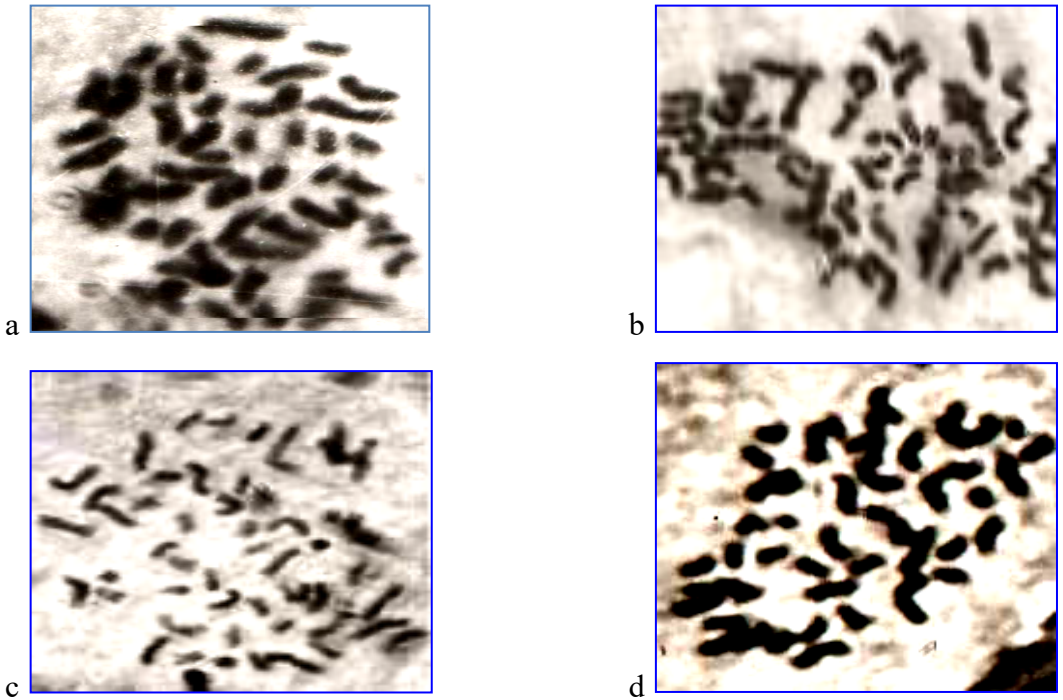
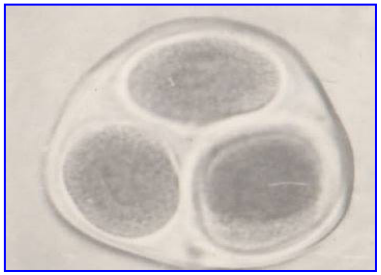
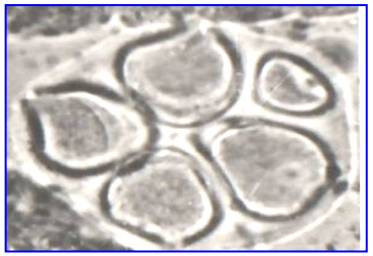


Figure 1. Photomicrographs of chromosome groups in somatic cell karyotypes of cotton complex intergenomic hybrid (a,d) and parental forms (b,c) plants: a) ♀ {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524}; b) ♂ Omad (*G. hirsutum* L.); c) ♂ Termiz 31 (*G. barbadense* L.) and d) {[F_1(*G. thurberi* Tod. × *G. raimondii* Ulbr.) × *G. arboreum* L.] × C-6524} × Omad.

a 

b 

c 

d 

Figure 2. Normal tetrad (a) and anomalies observed in complex intergenomic hybrid plants of cotton; triad (b); photomicrographs of pentads (c) and polyads (d) at 1000× magnification.