Study of DNA Synthesis Activity in High-Yielding Cotton Hybrids and Their Parental Forms

Mammadova Afet Dadash, Mammadova Naila Khurshud

Abstract**—**The purpose of this work was a comparative study of the DNA content in heterotic cotton hybrids and their parental forms. As a result of the research, it was found that the hybrids exceeded the original forms in terms of the content of DNA per leaf cell. So, for example, in the M-4 x AK-4-42 hybrid, the amount of DNA per somatic cell was 13.4 pg, in the maternal form it was 6.2 pg, in the paternal form - 12.0 pg. In the M-4 × C-460 hybrid, the content of DNA per leaf cell was 11.1 pg, in the parents, it was 6.2 pg and 5.0 pg, respectively

An increase in the absolute content of DNA in hybrids is observed due to the activation of all DNA fractions. For example, the hybrid M-4 x C-460 exceeds the maternal form in terms of the content of labile DNA by 2.2 pg, stable DNA by 2.3 pg, and residual DNA by 0.4 pg. The increase in DNA fractions in the hybrid in comparison with the paternal form was 2.7 pg, 2.8, 0.6 pg, respectively.At the same time, in heterotic hybrids of cotton, a decrease in the number of cells per unit area is noted, which indicates an increase in the size of the cells of the hybrid. In the M-4 x AK-4-42 hybrid, the number of cells per unit leaf area was 19 x 10^6 versus 24 x 10^6 and 21 x 10^6 in the original forms. At the same time, the mass of cells also increases in hybrids: the mass of one cell of the hybrid M-4 x AK 4-42 is 14.1 x 10^{-9} g, in parental forms - 10.7 x 10^{-9} and 12.8 x 10^{-9} g, respectively.

The study of the nature of DNA synthesis in cytoplasmic organelles revealed the activation of chloroplast DNA synthesis in hybrids compared to parents. The highest content of chloroplast DNA in comparison with parents was noted in the M-4 x 152F hybrid. The increase in the content of chloroplast DNA in comparison with the original forms in this hybrid was 76.9%.

Thus, the presented experimental data indicate that one of the causes of heterosis is the high absolute content of DNA per somatic cell in the leaf. An increase in the absolute content somatic cell in the leaf. An increase in the absolute content of DNA in the tissues of hybrid plants can be caused by polyploidization of somatic cells and the formation of "duplicates" for individual gene blocks and genome loci. The increase in DNA fractions is apparently associated with the formation of numerous endoploid cells in the hybrid organism. The activation of chloroplast DNA synthesis in hybrids also noted, apparently, indicates that if the nuclear and chloroplast genetic systems of a plant cell function with a greater load than in the parents, then, as a consequence, a heterosis effect can be expected. The results of our studies confirm the hypothesis of nuclear-cytoplasmic heterosis.

Keywords**—** Heterosis, cotton, DNA, cell, chloroplasts

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I. INTRODUCTION

It is known that the use of heterotic seeds makes it possible to increase the yield of plants by an average of 20-50% compared to the original varieties or lines, and improves the quality of products.For example, rice super-hybrids obtained in recent years in China exceed the yield of the best traditional varieties by 15-20% and have a productivity potential of 12-15 t/h [14].

Heterosis in cotton is manifested both in interspecific and intraspecific crosses. Heterotic hybrids are characterized by vigorous development,a large number of large bolls and a high fiber yield.Heterosisis especially pronounced when crossing *G. hirsutum* L. x *G. barbadense* L. In some combinations, such interspecific hybrids give heterosis in fiber yield by an average of 30–35%, sometimes it reaches 70%.

The study of the genetic mechanisms of heterosis is one of the priority areas, which will make it possible to get closer to understanding their nature and thereby effectively control the ways of increasing plant productivity, which is an urgent fundamental and at the same time applied problem [2, 11, 12].

Currently, most researchers understand the term “heterosis” as the superiority of first-generation hybrids over the best parental form in terms of the degree of development of one or a set of traits (the phenomenon of increasing productivity, viability, growth of hybrids due to the inheritance of a certain set of alleles of various genes from their heterogeneous parents).Along with true heterosis, hypothetical heterosis is distinguished, in which the value of the trait of the hybrid of the first generation is greater than the average value of the original forms [3, 4].

The phenomenon of heterosis covers many areas of biological processes and is considered in evolutionary, genetic, ontogenetic, functional and molecular biochemical aspects. In functional terms, there are reproductive, adaptive, selective heterosis and “luxurant”, that is, heterosis, which manifests itself in the powerful development of the vegetative mass of plants [7]. These types of heterosisare based on interallelic, intergenic, and plasma interactions, which can be functionally represented as processes of gene stimulation, compensation, and gene dose effect [5].Information about the structure and functional activity of the genome and plasmon of heterotic hybrids and their parental forms is especially valuable in revealing the nature of heterosis.

The DNA of higher plants is heterogeneous.This can be judged by molecular weight, nucleotide sequence, physicochemical properties, as well as the nature of its relationship with proteins and the way it is packaged in cellular structures, primarily in chromatin. Molecular heterogeneity of DNA is undoubtedly associated with its genetic differentiation, structural heterogeneity - with the functional activity of the genome. A method for revealing structural heterogeneity is fractional extraction, which made it possible to separate plant DNA into labile, stable, and residual. A method for revealing structural heterogeneity is fractional extraction, which made it possible to separate plant DNA into labile, stable, and residual.

The literature describes examples of the so-called plastid or chloroplast heterosis [9].A number of authors recorded the manifestation of the so-called "mitochondrial" heterosis [8].

Since the prerequisites for heterosisare formed in all genetic systems of a hybrid plant, the aim of this study was a comprehensive study of the functional activity of DNA, taking into account its localization in various cellular structures (nuclei, mitochondria, chloroplasts) in order to elucidate their possible role in the formation of yield during heterosis.

II. MATERIAL AND METHODS

The study used heterotic hybrids of cotton (*Gossypium* L.) and their parental forms.

To study the quantitative content of total, labile, stable, and residual DNA, the method of stepwise fractional extraction was used [6].Mitochondria and chloroplasts were isolated by differential centrifugation.The content of DNA was counted per cell, the number of which was determined by the method of Brown in the modification of M.A. Alizade [1].

III. RESULTS AND DISCUSSION

An analysis of the existing data on heterosis gives reason to believe that its manifestation is largely associated with the regulatory mechanisms of the genetic apparatus.The effect of heterosis, apparently, basically involves the activation of a gene or a complex of genes and belongs to the category of phenomena directly or indirectly related to the mechanisms of genetic regulation of the development of traits in ontogeny [5].

The table presents data on the study of the synthesis of nucleic acids in heterotic hybrids of cotton and their parental forms per somatic cell of the leaf.As the results of the study showed, the content of DNA per cell in cotton hybrids exceeded the parental forms.So, for example, in the M-4 x AK-4-42 hybrid, the amount of DNA per somatic cell was 13.4 pg, in the maternal form it was 6.2 pg, in the paternal form - 12.0 pg.In the M-4 × C-460 hybrid, the content of DNA per leaf cell was 11.1 pg, in the parents, it was 6.2 pg and 5.0 pg, respectively.

An increase in the absolute content of DNA in hybrids is observed due to the activation of all DNA fractions.For example, the hybrid M-4 x C-460 exceeds the maternal form in terms of the content of labile DNA by 2.2 pg, stable DNA by 2.3 pg, and residual DNA by 0.4 pg. The increase in DNA fractions in the hybrid in comparison with the paternal form was 2.7 pg, 2.8, 0.6 pg, respectively.

TABLE I
DNA CONTENT IN HYBRIDS AND PARENTAL FORMS

Sample, hybrid	Content of DNA per cell, g.10^{-12}				cell mass x10^{-9}	The number of cells, x 10^6
	labile	stable	residual	total		
M-4	2,0	3,4	0,8	6,2	10,7	24
AK 4-42	3,8	7,2	2,3	13,3	12,8	21
M-4 x AK-4-42	4,3	6,1	3,0	13,4	14,1	19
M-4	2,0	3,4	0,8	6,2	10,7	24
C-460	1,5	2,9	0,6	5,0	7,5	31
M-4 x C-460	4,2	5,7	1,2	11,1	10,9	21

At the same time, in heterotic hybrids of cotton, a decrease in the number of cells per unit area is noted, which indicates an increase in the size of the cells of the hybrid. In the M-4 x AK-4-42 hybrid, the number of cells per unit leaf area was 19 x 10^6 versus 24 x 10^6 and 21 x 10^6 in the original forms. At the same time, the mass of cells also increases in hybrids: the mass of one cell of the hybrid M-4 x AK 4-42 is 14.1 x 10^{-9} g, in parental forms - 10.7 x 10^{-9} and 12.8 x 10^{-9} g, respectively.

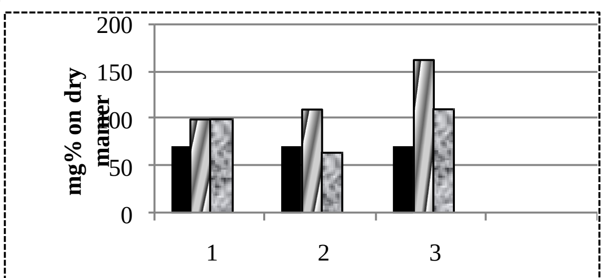
The DNA of labile chromatin is unsaturated with histones, has many free phosphate groups, and contains metastable, easily denaturable regions.. Due to this, DNA in the composition of labile chromatin is sensitive to acid hydrolysis.Labile chromatin is associated mainly with metabolic processes occurring in growing cells or in differentiated cells with an active physiological function. Chromatin labilizationis facilitated by factors that stimulate growth and metabolic processes in the body. Stable chromatin belongs to metaphase chromosomes and compact structures of interphase chromosomes, and is poor in nonhistoneproteins. DNA in stable chromatin is rich in histones and relatively poor in free phosphate groups. Stable chromatin is most found in dormant cells (seeds, dormant buds). An insignificant part of DNA (residual DNA) is bound in chromatin due to a special packaging in structures containing, in addition to proteins, RNA and lipids. In the residual chromatin, non-histone proteins were found that are common with labile chromatin, but absent in stable chromatin.

The genetic program of a plant cell is implemented not only at the level of the nuclear genome. An important role in the energy supply of the plant organism is played by mitochondria and chloroplasts, and the change in the genetic material of these cell structures during hybridization makes it possible to assess the energy intensity of the systems under study.The mechanism and regularities of the interaction of plasmogens with each other and with the nucleome during hybridization remain not yet fully elucidated.

Further studies were carried out by us to study the activity of DNA synthesis in cytoplasmic organelles in hybrids and their parents. We did not observe a certain regularity in the change in the DNA content in the mitochondria of hybrids in comparison with parental forms.A completely different picture was observed when studying the content DNA in chloroplasts.According to the content of chloroplast DNAall heterotic cotton hybrids exceeded their parents. Of the hybrid combinations studied, the highest activation of DNA synthesis in chloroplasts was noted for the hybrid M-4 x 152 F.The increase in the content of chloroplast DNA, in comparison with the original forms, in the hybrid M-4 x 152 F was 76.9%, in the hybrid M-4 x C-460 - 62.1%, in the hybrid M-4 x AK 4-42 - 15.2%. Apparently, in heterotic hybrids of this culture, the chloroplast genetic system contributes mainly to the total energy supply of the cell.

FIGURE.

DNA content in heterotic chloroplasts cotton hybrids and their parent forms



1. M-4 x AK - 4-42
2. M-4 x C-460
3. M-4 x152-F

There is evidence in the literature that hybrids have advantages over the parental forms in terms of the structure and functional features of the chloroplast apparatus; examples of the so-called “plastid heterosis” are described [13]. In these works, it was found that hybrids have a more perfect structure of chloroplasts.Hybridization leads to a change in the lamellar-granular structure of chloroplasts.A sign of the most active chloroplast in the hybrid is the compaction of lamellar systems due to an increase in the number of disks in the grains and grains in the chloroplasts.An opinion is expressed that the photochemical reaction of chloroplasts (Hill reaction and cyclic photophosphorylation), along with other physiological and biochemical indicators, can be used in assessing the source material in breeding for heterosis, and an increase in chloroplast activity and productivity in heterotic plants occurs due to favorable complementation of parental plastids forms during fertilization [10].

Thus, the presented experimental data indicate that one of the causes of heterosis is the high absolute content of DNA per somatic cell in the leaf. An increase in the absolute content of DNA in the tissues of hybrid plants can be caused by polyploidization of somatic cells and the formation of "duplicates" for individual gene blocks and genome loci.The increase in DNA fractions is apparently associated with the formation of numerous endoploid cells in the hybrid organism. The activation of chloroplast DNA synthesis in hybrids also noted, apparently, indicates that if the nuclear and chloroplast genetic systems of a plant cell function with a greater load than in the parents, then, as a consequence, a heterosis effect can be expected. The results of our studies confirm the hypothesis of nuclear-cytoplasmic heterosis.

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