

Physiological and Cytological Features of Fertilization, Formation of the Embryo and Endosperm During Interspecies Hybridization of Cotton

*Dilarom Daminova**, *Said-Akbar Rakhmankulov*, and *Khurshidbek Jalolov*

Cotton Breeding, Seed Production, and Agrotechnologies Research Institute, Tashkent, Uzbekistan

Abstract. The article presents the data of physiological and cytological studies on the study of the features of the process of fertilization, the formation of the embryo and endosperm in the distant interspecific hybridization of cotton. Normally, the influx of hormones that regulate the division of the endosperm occurs in the opposite direction of the movement of phloem and xylem nutrient flows, which cause the movement of division waves and end by the 8th-9th day with the cell formation of the nuclear endosperm, which occurs by delimiting the cytoplasm around the nuclei through the formation of chains of vesicles emerging cell walls, followed by the formation of cell walls. And in the process of formation of a distant interspecific endosperm, a massive multiple formation of abnormal nuclei occurs, which remain viable due to the total metabolism and only in the common cytoplasm of a multinuclear cenocyte. It was revealed that the imbalance of the chromosomal composition of the nuclei in the common nuclear coenocyte leads to the death of its nuclei before the stage of cell formation and, therefore, when interspecific embryos switch to endosperm nutrition, they lose it, which blocks further growth and development of interspecific embryos inside the forming seed. Key words: cotton, distant interspecific hybridization, embryo, endosperm, pollen tube, synergid, nucellus, coenocyte.

1 Introduction

At the present stage in cotton, as a result of intensive selection, the species genetic potential of close allotetraploid species *G. hirsutum* L. and *G. barbadense* L. has been practically used, in which many valuable genes of wild relatives have been lost during long-term cultivation. As proved by theoretical and practical works on cotton [1-11], wild and ruderal forms of cultivated species and primordial wild species of cotton have many traits that are missing in cultivated varieties due to genes and gene complexes, such as precocity, drought resistance, deciduousness, immunity to diseases, resistance to agricultural pests, uniquely high fiber quality (strength, fineness, silkiness) and etc. [12-15].

* Corresponding author: daminovad19860@gmail.com

To date, the presence of economically valuable genes and gene complexes of resistance to various biotic and abiotic extreme environmental factors in known wild diploid cotton species has been proven. However, during interspecific hybridization of allotetraploid cultivated cotton species with wild diploid species, even in the case of obtaining completely viable hybrid plants of the first generation, as a result of uncoordinated interaction of the genes of distant parental species, obstacles arise in the distribution of chromosomes in meiosis, which leads to the formation of gametes with an unbalanced the number of chromosomes and there is a reduced viability of hybrids or their complete sterility. Meiosis disorders that occur in interspecific cotton hybrids are due to the manifestation of the incompatibility barrier.

In a broad concept, the barrier of incompatibility is the impossibility or obstacles to the unification of gametes of organisms of different species (subgenera, genera, families) when they are crossed, developed in the process of evolutionary divergence or interspecific isolation, for a period (according to paleontological data) of at least 2-4 million years.

In connection with the above, the purpose of these studies was the cytological study of the characteristics of the growth of pollen tubes, the formation of the embryo and endosperm in the parent species *G. hirsutum* L. and in the formation of distant interspecific hybrid seeds of the F0 generation.

2 Materials and methods

The experiments were carried out at the Research Institute of Selection, Seed Production and Agricultural Technology of Cotton Growing. Crossings between distant heterochromosomal species were carried out according to the generally accepted method. The treatment of ovaries during multichromosomal hybridization with solutions of phytohormones was carried out 2 days after pollination [16]. For cytoembryological studies, 1-day after pollination, the pistil columns were fixed in 70% ethanol, and 2-, 3- and 4-day-old ovules were fixed in Carnoy's fixative (6:3:1) according to Z.P. Pausheva (1986). Posting of the fixed material was carried out according to the method generally accepted in cytoembryology [17]. The preparations were examined under microscopes MBI-3, MBR-3. Microphotographs were taken using microphotographic attachments MFN-11 and MFN-12.

3 Results and discussion

It is known that the embryo sac, embryo and endosperm of cotton develop in a close environment of the tissues of the ovule inside the ovary. In practice, they are not available (without causing serious wound damage) for cytophysiological studies. However, they are quite convenient material for cytoembryological studies.

These studies were carried out according to the methodology indicated in the section "Materials and Methods of Research". On the parent SS variety Bukhara-102 and its hybridization with wild 26-chromosomal cotton species *G. australe*, *G. nelsonii*, *G. bickii*, *G. stocksii*, *G. incanum*, *G. davidsonii* and *G. laxum*.

As shown by numerous (including ours) observations during distant interspecific hybridization of allotetraploid species with wild diploid cotton species, the growth of foreign genome pollen tubes and the process of fertilization occur at a slower pace and in single ovules 3-4 days later than in the parent variety. During interspecific hybridization, the patterns of entry of pollen tubes into the micropyle of ovules and fertilization were observed even in fallen 6-8-day old ovaries.

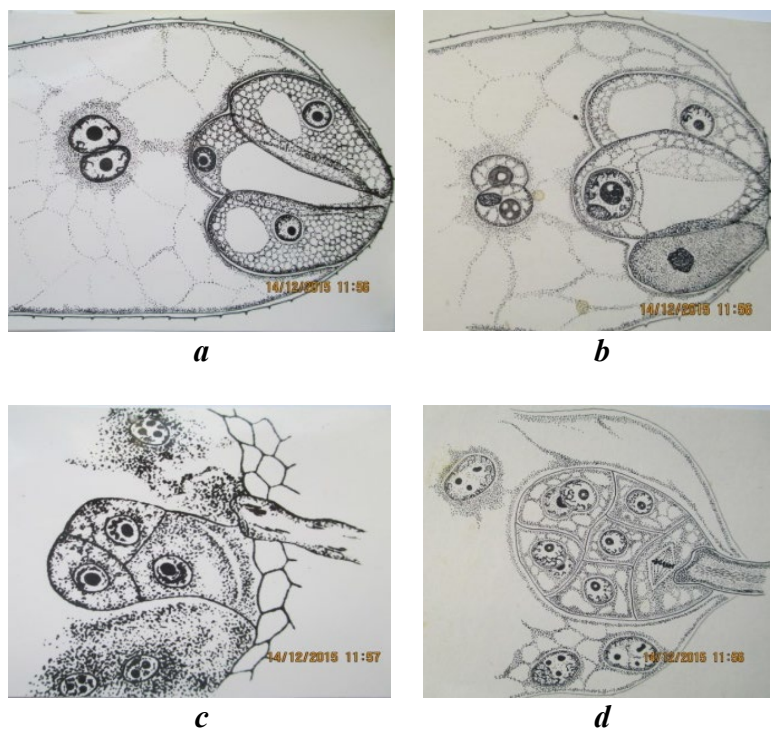


Fig. 1. Fertilization and the initial stages of the formation of intersubgenal embryo (magn. 7 x 40) in the *G. hirsutum* x *G. bickii* variant:

a– mature embryo sac; *b*– double fertilization (sperm and cloudy synergids are visible); *c*– the initial stages of embryo formation, the remains of the pollen tube and the first endosperm nuclei; globular embryo, remains of pollen tube and endosperm nucleus.

However, at the same time, there were no noticeable violations, except for slowing down the process of fertilization (Fig. 1 a, b). 2–3 days after foreign genomic fertilization, the zygote began to divide, forming a two-celled embryo, the lower cell of which formed a suspension-like structure (Fig. 1c). The upper cell by 8–10 days after fertilization formed an undifferentiated, multicellular, spherical embryo (Fig. 1d).

Further, the globular embryo soon after the formation of initial cells (apical, root and 2 cotyledon leaves) acquired a triangular fan shape. Physiologically, fertilization took place due to the absorption of nutrients from the destroyed pollen tube. The zygote then consumed nutrients from the cytoplasm of the embryo sac.

The formation of a multicellular pre-embryo globular embryo, up to the beginning of differentiation into the rudiments of cotyledon leaves and the rudimentary root, and the next stage of the heart took place due to the functioning of the auxiliary cell of the embryo sac (the remaining living synergid), which increased in size, forming a mesh structure.

However, the functioning of the synergid was ephemeral, and, by the 8-10th day after fertilization, it had already degraded. This process is normal both in control and in interspecific hybridization of cotton. In parallel, at the same time, after fertilization in *G. hirsutum* L. in the chalazal part of the embryo sac, the formation of the fertilized central nucleus (formed by 2 polar nuclei) takes place - the ephemeral tissue that feeds the embryo - the endosperm. It should be noted that the developing embryo is overgrown with a developing endosperm sac, which, in turn, is surrounded by a thin, dense shell of macrosporangium that increases with growth; dense and thickened in the middle part, thin at the micropylar and chalazal ends. Therefore, the penetration of nutrients from the

nucellus into the endosperm sac (and then to the embryo) is possible only from the side of the micropyle and chalase.

Judging by the anatomical structures framing the developing endosperm, xylem elements of the conducting system (according to Chailakhyan, 1988) approach the chalazal end of the ovule, supplying water and minerals dissolved in it, as well as kinins synthesized in the roots, to the endosperm [18; 19]. The haustorial protrusion on the chalazal side that nourishes the endosperm covers the hypostasis, which performs the function of supporting the soft tissues of the ovule and serves as a store of kinins.

From the micropylar side, the endosperm receives phloem nutrition through the nucellus tissues, consisting of sugars, amino acids, and vitamins (including phytohormones) [20], synthesized in the green parts of the flower, leaves, and formed during photosynthesis in chloroplasts.

According to studies, the endosperm, in which the embryo is immersed, is a "natural sterile chamber", which provides a very rapid reproduction of the endosperm nuclei and the necessary formation of a special nutritious endosperm fluid consumed by the developing embryo.

The structure of the actively dividing cotton endosperm is initially a nuclear coenocyte (Fig. 2), in which numerous nuclei are immersed in the common cytoplasm, and which is preserved from the beginning of divisions of the fertilized central nucleus until the 8-10 day old nuclear coenocyte, after which the endosperm becomes cellular.

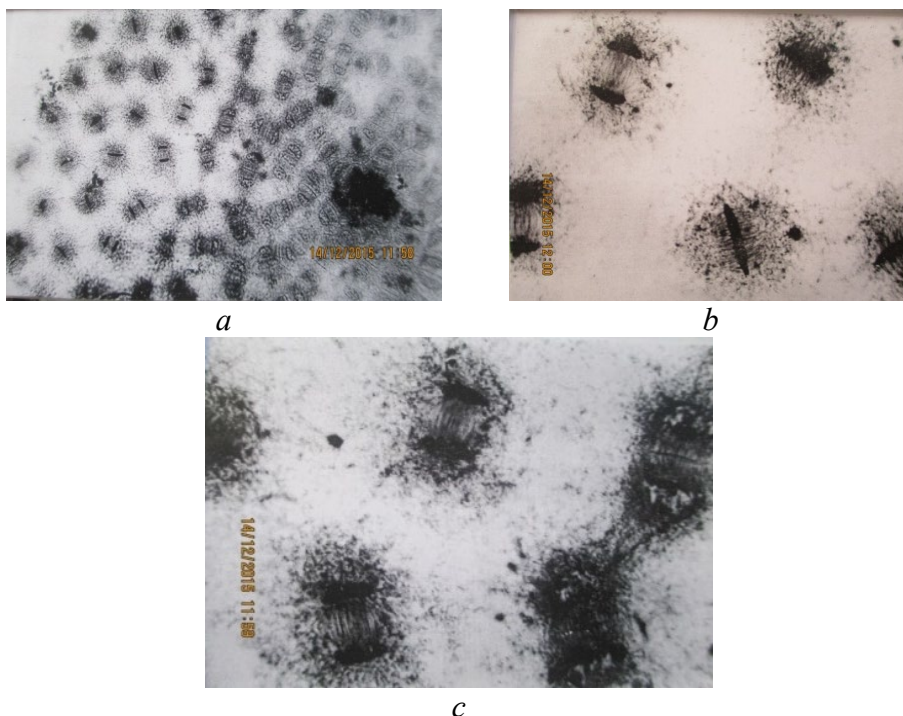


Fig. 2. Phases of division waves in the parent species *G. hirsutum* L. (variety Bukhara-102) *a*– general picture of the wave of nuclear fission (view from the chalazal part of the endosperm) (magnification 7x40); *b*– metaphases and anaphases of the wave of divisions (sw. 7x90) of the nuclei of the endosperm; *c*– anaphase and telophase waves of divisions (magnitude 7x90) of endosperm nuclei.

The main feature of the cotton endosperm at the stage of nuclear cenocyte is the division waves of numerous nuclei, the zones of which pass through the endosperm in

regular, strictly geometrically oriented (circular to the longitudinal axis) synchronized by the simultaneous passage of the phases of mitotic divisions of nuclear chromosomes (Fig. 2). With the help of fission waves, the number of nuclei increases exponentially. In just 8-10 days, there are 8 such waves, increasing (normally) the number of nuclei from 1 to 256. According to the number of nuclei formed, the size of the nuclear endosperm, the megasporangium membrane and the volume of the endosperm fluid also increase.

According to the studies [20; 21], the endosperm is a specific nourishing tissue of the embryo, belonging to the category of hormone-dependent tissues, in contrast to the embryonic tissues.

From the classical studies of R.G. Butenko (1986) [22], it is also known that the entry of plant cells into the division process is under bifactorial double hormonal control: first, auxins prepare the nuclei for division (the permeability of membrane pores increases, specific RNA is synthesized, etc.); only in auxin-prepared nuclei, cytokinins are triggers - a "trigger" that activates the genes responsible for the passage of divisions; Individually, each of these phytohormones cannot cause plant cell division [23-28].

Therefore, the wave of mitotic nuclear fission in the endosperm takes place only when the optimal ratios of the concentrations of phytohormones entering the nucellus, and from it into the endosperm, together with nutrients (from the mother's body), which is 3:1 (auxins : kinins) are reached. This optimal hormonal ratio evenly moves in a ring along the long axis of the nuclear endosperm, forming a wave of divisions.

Considering the extremely geometrically correct patterns of moving fission waves in the nuclear cenocyte of the cotton endosperm and the evenness of the zones (Fig. 2) of numerous patterns of divisions in phases (obviously corresponding to the gradients of division hormones - division rings) - physiologically shows the specificity of the nutrition of the cotton endosperm, in which:

- the supply of nutrients through the thickened lateral parts of the megasporangium shell is not possible;

- the influx of plastic nutrients with auxins from phloem elements from chlorophyll-containing organs and from the chalazal conductive bundle (through elongated thin-walled cells) occurs by osmosis;

 - through the chains of nucellus cells into the endosperm from the micropillar end;

 - in this way, the maximum intake of the hormones auxins and gibberellins, which are synthesized during the daytime of active photosynthesis, is carried out;

 - the influx of water with minerals, trace elements and dissolved cytokinins, the maximum of which occurs at night from the conductive bundle also by osmosis, bypassing the hypostasis, through the endospermal haustorium from the chalazal end of the endosperm.

In the endosperm, these two oppositely directed flows of phytohormones that control nuclear fission (at different times of the day passing maximum concentrations) reach the optimal ratio of concentrations for the preparation and start of divisions; which are manifested at the level of the wave of divisions of the nuclear cenocyte. Thus, the initial preparation of nuclei for fission by auxins begins at the micropillar end; the usual distribution of division waves corresponds to the flow of cytokinins from the chalazal end.

After each passage of a wave of divisions (usually one per day), the free nuclei of the endosperm cenocyte fall into the state of interphase. Since division hormones are synthesized in leaves and roots at different times of the day, there is a physiological dependence of the regular daily rhythms of division waves, the interphase and fission state of the coenocytic endosperm nuclei, on the daytime photosynthetic activity of the leaves and the nighttime root activity.

Thus, based on the current level of knowledge about the bifactorial (auxins, cytokinins) hormonal conditioning of nuclear divisions in cotton plants, with their optimal ratio; and

comparison of cytoembryological and anatomical features of the structure of cotton ovules, the features of nutrition and the formation of the embryo sac and endosperm are revealed. At the same time, the influx of hormones that regulate the division of the endosperm occurs in the opposite (at different times of the day) direction of movement of phloem and xylem nutrient flows, which cause the movement of division waves and normally end by 8-9 days (after fertilization) with the formation of nuclear endosperm cells, which occurs by delimiting the cytoplasm around the nuclei through the formation of chains of vesicles of emerging cell walls, followed by the formation of cell partitions. After the endosperm becomes cellular, it begins to actively store nutrients in the form of oil, sugar and starch and actively nourish the developing embryo at the subsequent stages of elongation and folding of the cotyledon leaves.

The above patterns of formation and functioning of the endosperm are observed in the control mother plants *G. hirsutum* L.

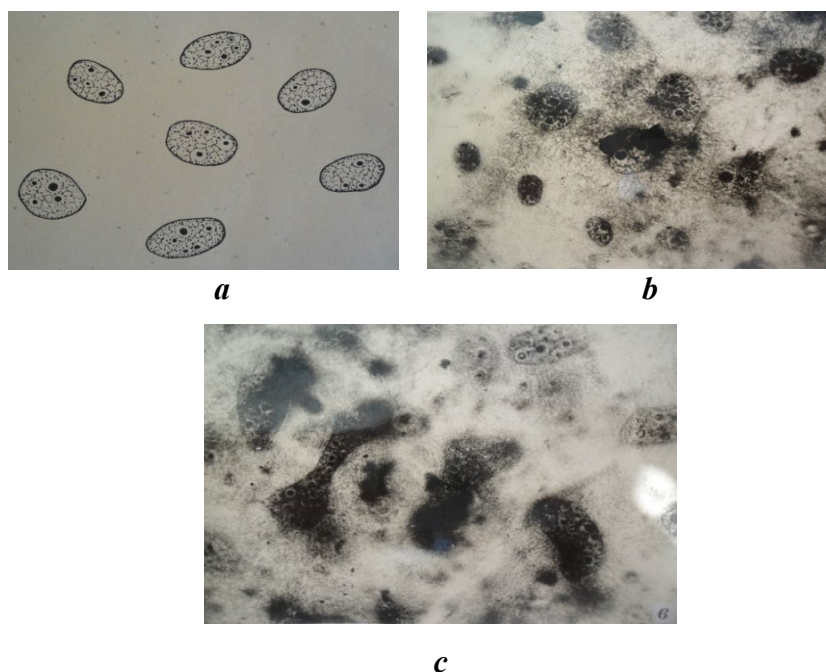


Fig. 3. Normal and abnormal endospermal cotton kernels (sw.7x60) **a** – nuclei of normal endosperm of the original maternal species *G. hirsutum* L. **b**, **c**– nuclei of dying interspecific endosperm in F0 *G. hirsutum* x *G. australe*

In a different way, the endosperm is formed in interspecific hybrids by the 8th–10th day (Fig. 3). Since during double fertilization during interspecific hybridization, the endosperm is formed by the allotetraploid set of maternal chromosomes and the haploid set of foreign genomic sperm, each chromosome of 13 is univalent. Therefore, in each of the eight successive mitotic divisions, each foreign genomic paternal univalent chromosome (having a different rate of spiralization, conjugation and composition of genes than the maternal chromosomes) can either: split into 2 chromosomes, with the passage of one to each of the new nuclei; either both chromosomes go to one of the nuclei, and the other does not receive a single chromosome; or the univalent chromosome does not divide at all and goes to one of the forming nuclei. In this case, nuclei of the interspecies endosperm, abnormal in their chromosomal composition, are formed, which can function only in the common cytoplasm. Therefore, already at the beginning of the stage of separation of the endosperm nuclei by

cell partitions in the control, each individual nucleus of the interspecific hybrid becomes unviable.

4 Conclusion

Thus, from the given cytological pictures of the formation of abnormal endosperm nuclei (Fig. 3) it can be seen:

- in the process of formation of a distant interspecies endosperm (with each of the eight successive waves of mitotic divisions), there is a massive multiple formation of abnormal (aneuploid and hyperploid) nuclei; due to which endosperm nuclei remain viable due to the total metabolism and only in the common cytoplasm of a multinuclear cenocyte;
- when the interspecies endosperm enters the phase of cell formation, its abnormal nuclei are unable to function normally when nutrients enter through cell pores and plasmodesmata and, moreover, to carry out intensive synthesis of reserve nutrients;
- imbalance of the chromosomal composition of the nuclei in the common nuclear cenocyte leads to the death of its nuclei before the stage of cell formation;
- consequently, when interspecific embryos switch to endosperm nutrition, they lose it, which blocks further growth and development of interspecific embryos inside the developing seed.

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