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Cytological Features of Male Gametophyte Formation from Distant Hybrids *Pyrus* X *Malus* and *Ribes* X *Grossularia*

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Abstract
The authors have determined anomalies in the formation of male gametophyte in pear-apple and currant-gooseberry hybrids, leading to uneven distribution of the genetic material among the poles of meiocytes' division and disruption of chromosome balance in the cells of pollen. However, the formation of part of morphologically made pollen grains occurs, allowing the use of studied hybrids in further breeding work as pollinators.

Keywords: microsporogenesis, male gametophyte, intergeneric hybrids, *Pyrus*, *Malus*, *Ribes*, *Grossularia*.

Introduction

One of the main problems in the modern horticulture is creating highly adaptive varieties of fruit and berry crops with a set of economically valuable traits. To this end, the use of the method of distant hybridization allows to highly efficiently obtain complex versatile changes in the characteristics and properties in plants [1-3].

A characteristic feature of distant hybrids of fruit and berry crops is their reduced fertility, which manifests itself, depending on the specific genotype, in a wide range - from completely sterile to partially fertile forms [4-7].

To develop more efficient methods of coping with this negative phenomenon, it is necessary to know the peculiarities of generative organs' formation, pollination, fertilization, and other processes. In this respect, in the present work the authors studied the process of microsporogenesis in intergeneric hybrids with the aim of obtaining data about the possibility of using them as source parental forms in further breeding.

Studying meiosis in remote amphihaploid and amphidiploid hybrids has great scientific and practical importance. Studying the process of chromosomes' conjugation during the first meiotic division is one of the most efficient methods of establishing phylogenetic relationships between the species that were parent forms for hybrids [8]. This genomic analysis allows to determine the presence of partially affined (homeologous) chromosomes in genetically distant source types that belong to various species, or even genera. Cytological analysis of meiotic chromosome associations in hybrids is important, since such a study can set the balance among the genotypes of these hybrids in the formation of germ cells and further use of the studied forms in the breeding process.

Materials And Methods

The authors studied the cytological features of distant pear-and-apple hybrid 01 (*Pyrus communis* L. x *Malus domestica* Mill.) bred by S. F. Chernenko and the currant-and-gooseberry amphidiploid SKG No. 5-4 (*Ribes nigrum* L. x *Grossularia reclinata* Mill.) bred by E. Kip.

The buds from the natural conditions in the spring were temporally fixed in Carnoy's fixative. The progress of meiosis was studied on squashed aceto-hematoxylin preparations of anthers [9]. The preparations were viewed under a Carl Zeiss Jenamed microscope, pictures were taken with digital camera DCM-500 with Scope Photo software. The data were statistically processed in Microsoft Excel.

Results

As a result of cytological study of pear-and-apple hybrid 01, a number of violations in the process of meiotic division had been registered (Fig. 1).

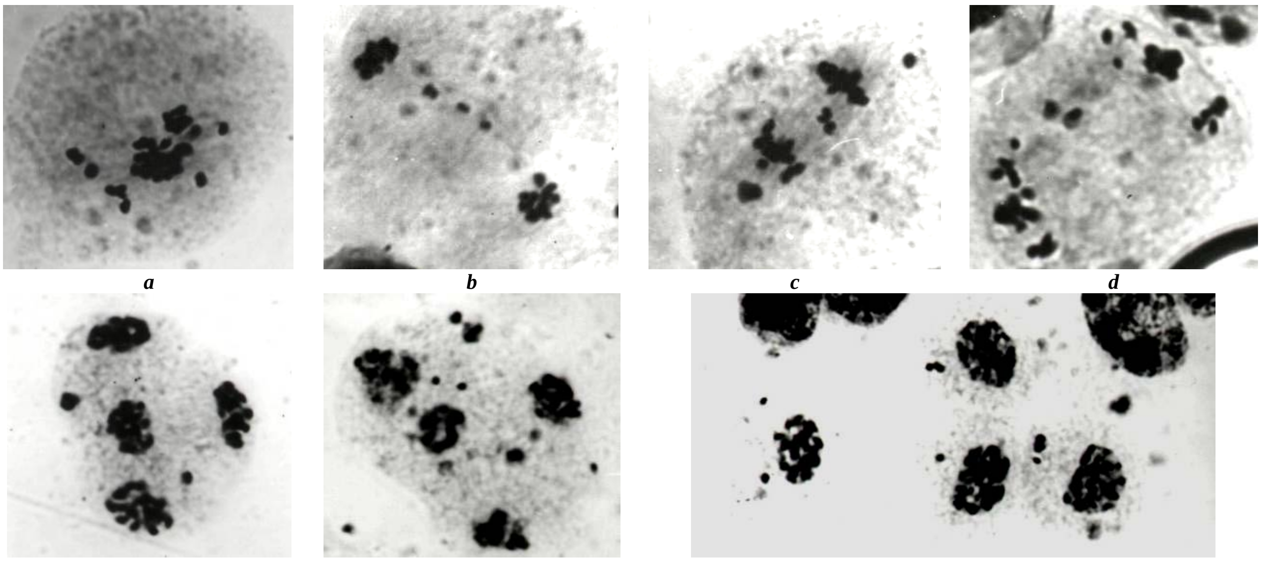


Fig. 1. Disruptions in the meiosis of pear-and-apple hybrid 01: *a* – outbursts of chromosomes in metaphase I; *b* – retardation of chromosomes in anaphase I; *c* – retardation and outbursts of chromosomes in anaphase I; *d* – retardation and outbursts of chromosomes in anaphase II; *e* – formation of micronuclei in telophase II; *f* – formation of hypocells; and *g* – micronuclei in microspores

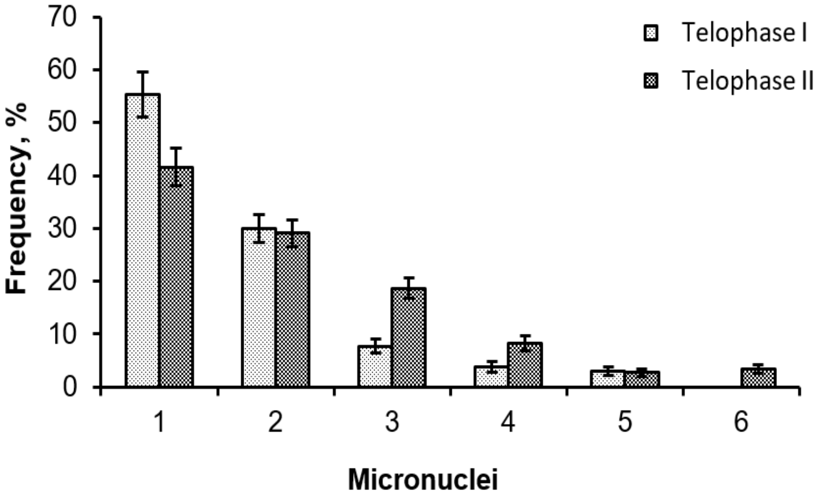


Fig. 2. Micronuclei formation in pear-and-apple hybrid 01 in the telophase of first and second meiotic divisions

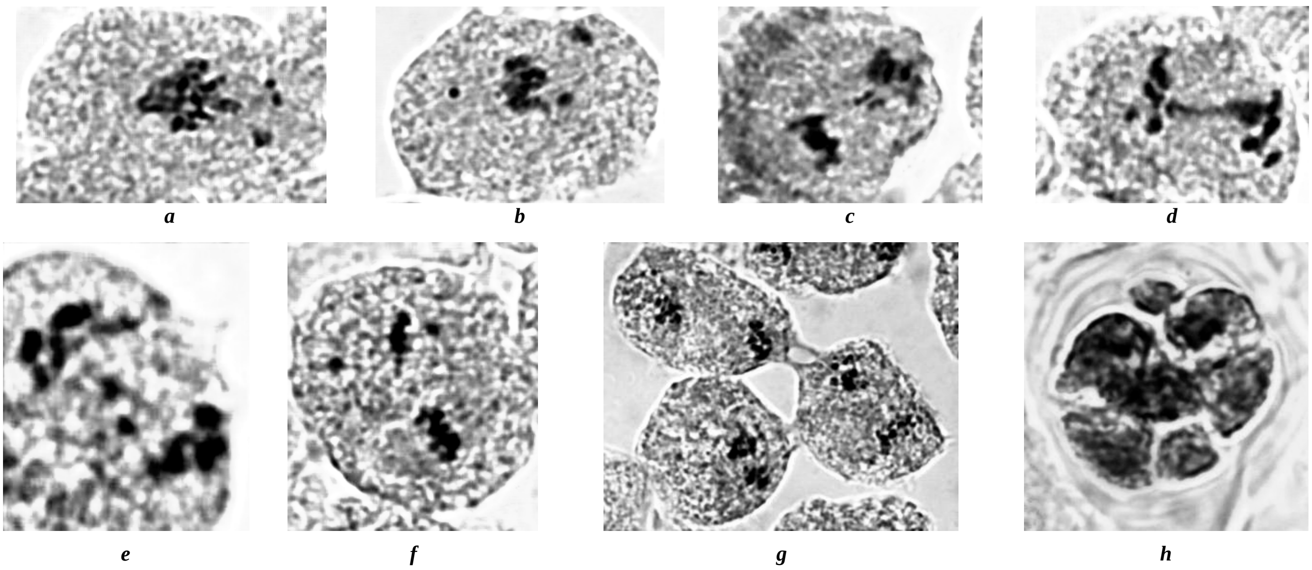


Fig. 3. Disruptions in the meiosis of currant-and-gooseberry hybrid SKG No. 5-4: *a* – outbursts of chromosomes in metaphase I; *b* – advancing and outbursts of chromosomes in anaphase I; *c* – retardation of chromosomes in anaphase I; *d* – chromosomal bridge in late anaphase I; *e* – formation of micronuclei in telophase I; *f* – outbursts of chromosomes in anaphase II; *g* – cytomixis among several meiocytes; and *h* – formation of a polyade

At the earliest stages of chromosomes' condensation in prophase I, namely, zygoneme, nucleoreticula in homologous chromosomes were connected pairwise. However, in this period they were strongly intertwined, which made it extremely difficult to study the degree of their homology. To observe possible anomalies of chromosomes' conjugation, the period of diakinesis, when anomalies became noticeable, was the best. At the stage of diakinesis, conjugation with each other was disrupted in part of chromosomes due to the genetic differences between the genomes of apple and pear, which resulted in the formation of univalents. Their average number per cell was 2.4±0.6, while the number of bivalents was 15.5±1.2.

In the anaphase of the first division, disruptions of the synchrony of chromosomes' divergence to the poles were observed, manifested by their premature leaping ahead (62.1±5.6% of the total number of disruptions at this stage) and retardation (25.2±3.0%). Most often, 2 to 3 chromosomes with disrupted anaphase movement were observed. A characteristic feature AI in pear-and-apple hybrid 01 was the formation of chromosomal bridges (16.3±1.7%).

As a result of numerous retardations, leaping ahead and outbursts of chromosomes into the cytoplasm at stages of MI and AI in the telophase of the first division, the formation of micronuclei was observed as a consequence, containing different numbers of chromosomes (up to 4x), 1-2 in most cases. The number of micronuclei at this stage varied between 1 and 5 (Fig. 2).

In pear-and-apple hybrid 01, homeotypic division, similar to the heterotypic one, proceeded with disruptions in metaphase II, divergence of chromatids occurred unevenly, like in equational division, whereby their numbers in each group were different.

The anaphase of the second division was characterized by outbursts of many chromatids outside the spindle apparatus (30.9±2.4%), the presence of chromosomal bridges (5.8±0.9%), and nondisjunction of chromatids from the equatorial plane (6.4±1.0%).

At the stage of telophase II, in addition to the normal formation of four groups of chromosomes, one or two separate chromosomes were observed in the cytoplasm, as well as groups of chromosomes, which by completion of stage TII had formed micronuclei, the number of which might reach 6 (Fig. 2).

Studying tetradogenesis in pear-and-apple hybrid 01 has shown that, despite numerous anomalies at various stages of meiotic division, some tetrads were formed with full-fledged cores of the same size (21.3±2.0% of the total number). However, tetrads with nuclei of different volume predominated, amounting to 36.1±3.7%. Formation of polyades was common for forms with a large number of micronuclei in TII; however, in this hybrid only 2.9±0.4% of pentads and 1.6±0.3% hexads were found, which could be explained by lysis in most genetically unbalanced mother cells of the microspores at the early stages of tetradogenesis. A characteristic feature of this hybrid was the formation of dyads (3.6±0.4%) and triads (13.3±1.5%). This suggested potential possibility of the hybrid forming unreduced gametes (Fig. 5a, 6a), which further had been confirmed in studying the dimensions of formed pollen grains.

Studying the process of microsporogenesis in distant amphidiploid hybrid SKG No. 5-4 (2n=4x=32) showed the presence at all stages of meiosis of cells with various anomalies, the number of which increased in equational division. The most frequent deviations from the norm observed in this form were disruptions in the rate of chromosomes' divergence in anaphases I and II, formation of micronuclei in telophase I and II, cytomixis in all stages (Fig. 3).

In the metaphase of the first division, a small number of violations (14.9±2.9%) was observed, which were dominated by outbursts of univalents (71.4±3.7% of the total number of disruptions).

In anaphase I, the main anomalies were premature advancing and outbursts of chromosomes, which made 75.4±2.1% of the total number of disruptions at this stage, and retardation of chromosomes (14.1±1.7%). Chromosome bridges (4.2±0.9%) and cytomixis pictures (2.8±0.8%) were observed.

In telophase I, which was the final reduction stage of meiotic division, chromosomes' outbursts into the cytoplasm were observed in part of cells (35.2±2.3%), which resulted in micronuclei formation (Fig. 4). At this stage, 2.7±0.8% of disruptions were cytomixes.

In metaphase II, the main disruptions were outbursts of chromatids outside the equatorial plate (78.6±3.1%).

In the anaphase of the second division, advancing and outbursts of chromatids were noted (52.4±3.8%), retarded and nondiverged to poles chromatids made 9.5±2.2%.

Outbursts of chromatids into the cytoplasm were recorded in telophase II in the amount of 42.8±3.3%, and most of them formed 1 to 2 micronuclei (Fig. 4).

The specific violations noted in currant-and-gooseberry amphidiploid SCG No. 5-4 at all stages of meiosis were pictures of multiple cytomixis among several meiocytes simultaneously, which greatly increased in course of equational division – from 2.8±0.8% in anaphase I to 32.3±3.6% in anaphase II.

At the stage of tetradogenesis, anomalies were mainly hexades and pentades, which correlated with more frequent formation of 1-2 micronuclei in telophases I and II. Dyads, triads and polyades were observed much less frequently than the extreme variants of tetradogenesis disruptions, which was consistent with the general biological law of the normal distribution.

These violations collectively resulted in the formation of abnormal tetrads, and consequently, formation of morphologically nonhomogeneous pollen (Fig. 5).

Analysis of the variation curves of pollen grains' diameter showed the morphological nonhomogeneity of the resulting pollen formed from hyper - and hypomeiocytes, which resulted from disruptions during microsporogenesis at various stages.

As seen in Figure 6a, the curve of sterile pollen of pear-and-apple hybrid 01 was offset to the left relative to the fertile one, due to the presence of small, morphologically incomplete pollen grains formed from hypocells. The curve of pollen stained with acetocarmine had polymodal nature. Of the total number, there was a size group of pollen grains with the diameter ranging between 45 and 52 µm, while the average value of this characteristic for all fertile pollen was 32.4±1.0 µm. This allowed considering it diploid (2n), given the ratio between the average size of pollen at the two peaks of the curve was 1:1.8. The total amount of nonreduced pollen was 11.4%, which was close to the total number of dyads and triads in tetradogenesis.

The range of pollen grains' diameter variation in currant-and-gooseberry hybrid SKG No. 5-4 was from 32 to 56 µm, the average diameter being 41.4±0.3 µm (Fig. 6b).

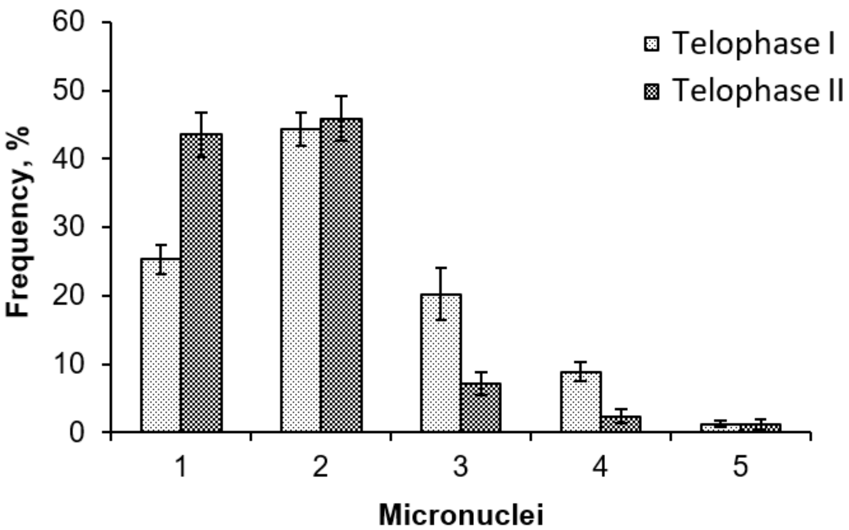
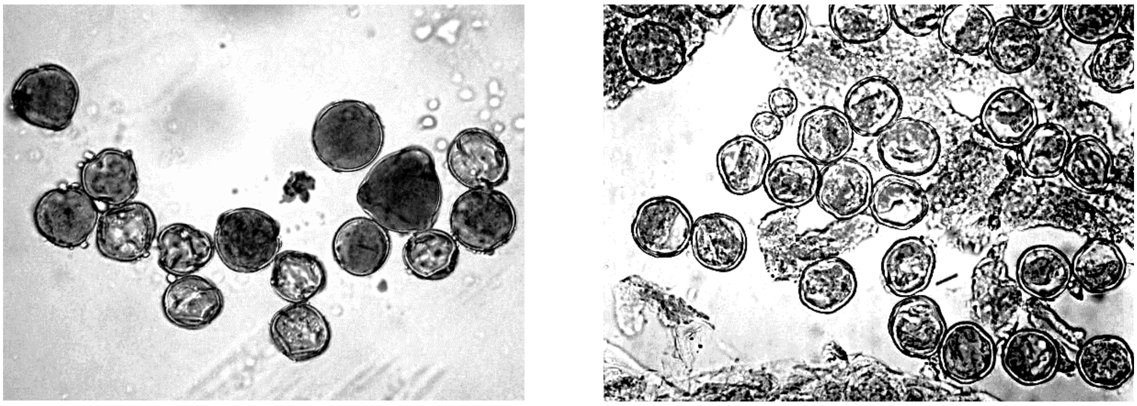
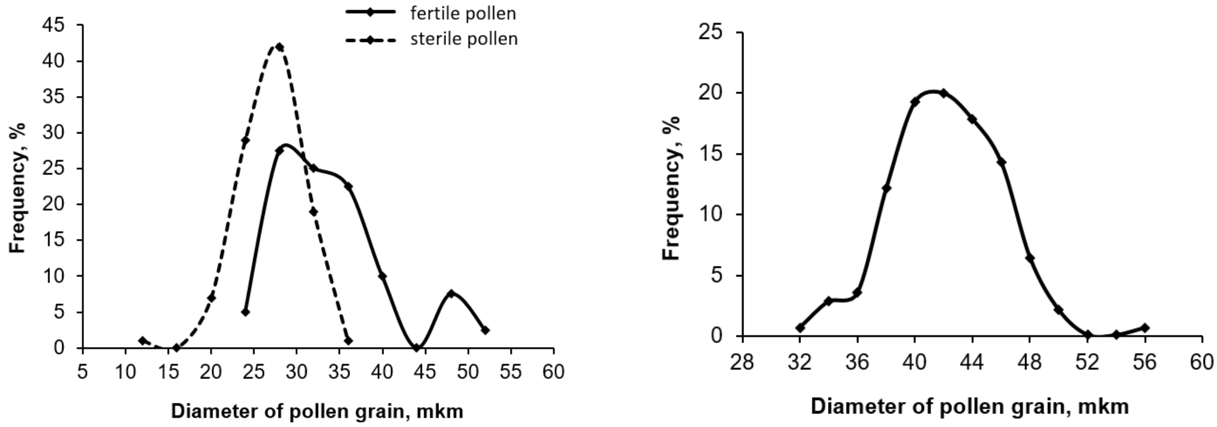


Fig. 4. Formation of micronuclei in currant-and-gooseberry hybrid SKG No. 5-4 in the telophase of the first and the second meiotic division



a *b*

Fig. 5. Pollen of pear-and-apple hybrid 01 (*a*) and currant-and-gooseberry hybrid SKG No. 5-4 (*b*) stained with acetocarmine



a *b*

Fig. 6. Variation in the size of pollen of pear-and-apple hybrid 01 (*a*) and currant-and-gooseberry hybrid SKG No. 5-4 (*b*)

Conclusions

Cytological studies have shown a number of deviations during the formation of the male gametophyte in pear-and-apple hybrid 01 and currant-and-gooseberry amphidiploid SKG No. 5-4. Disruption of normal chromosome conjugation manifested in the formation of several univalents instead of normally bivalents should be regarded as the main prerequisite for meiosis abnormalities.

However, the general nature of anomalies in the studied intergeneric hybrids *Pyrus* x *Malus Ribes* x *Grossularia* can be clearly seen in the presence of individual specific deviations in the meiotic process. In these forms, the authors noted disruptions of the normal rate of chromosomes' divergence in anaphase I and II, outbursts of chromosomes outside the spindle apparatus, formation of micronuclei in telophase I and II, which together led to uneven distribution of genetic material between the poles of meiocytes' division and disruption of chromosome balance in the cells of pollen. As a result, gametes are formed with the number of chromosomes not corresponding to the haploid set of chromosomes for these forms.

Along with that, in addition to abnormal sporades, normal tetrads are formed producing full-fledged pollen grains, which allows the use of these genotypes in further breeding work.

The valuable feature of pear-and-apple hybrid 01 is its ability to form more than 10% of nonreduced pollen from morphologically complete one, which can greatly contribute to obtaining valuable polyploid genotypes of pomaceous fruit crops provided the evidence-based choice of the mother form and participation of this form in hybridization as a pollinator.

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