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Cytomixis During Microsporogenesis in *Cerasus fruticosa* Pall

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ABSTRACT

The migration of chromatin between cells in microsporogenesis in *Cerasus fruticosa* Pall occurs through plasmodesma or by cell fusion. Cytomixis is detected in 13.0% of anthers. Cytomixis often occurs at the earliest stages of chromosome condensation in prophase I (leptonema, zigonema) and develops as a full cell fusion. This anomaly begins with the formation of 1 - 3 cytoplasmic canals between meiocytes. Then the canals begin to increase in size. Both cells unite. The cytomixis consequences occur at different stages. At the meiosis final stages the chromatin that migrates into the recipient cell is defined as micronuclei or cytomixis polyads. The transfer of nuclear material can occur between two at most cells. The limiting factor of chromatin multiple multidirectional migration is the microsporocytes contact density in the anther. The chromosomes migration causes sporogenic cells sterilization. Cytomixis has been observed during microsporogenesis in tapetal anther cells.

Key words: Cytomixis, microsporogenesis, meiosis, *Cerasus fruticosa Pall*.

INTRODUCTION

The outthrust of chromatin and its transfer from a microsporocyte nucleus to the next microsporocyte cytoplasm is known in many plants (Mageshvari, 1954; Kamra, 1960; Tompson, 1962; Thomas, 1964; Sherudilo, 1964; Shkutin, 1969; Romanov, Orlov, 1971; Kozlovskaja, Khvostova, 1972; Turovtseva, Luchnikova, 2001; Yandovka, 2004a, 2004b; Saggoo *et al*., 2011; Mursalimov *et al*., 2012). There are several definitions of cytomixis in the literature: “The fusion of chromatin from two tissue cells” (Gates, 1911); “The transfer of an unstructured chromatin drop from one cell to another” (Kihara, Lilienfeld, 1934); “The exchange of cytoplasm with cell organelles by cells, the migration between microsporocytes of a cytoplasm part with chromosomes, their fragments” (Romanov, Orlova, 1971); “The transfer of the cytoplasm with the nucleus or its part to the next cell” (Orlova, 1994).

The application of different staining methods (Sparrow, 1947), electron microscopy (Orlova, 1994) and autoradiography (Takats, 1959) has shown that the bodies emerging from the cells contain DNA.

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There is no consensus about the nature and significance of the cytomixis. Some authors have noted the transfer of DNA from the tapetum to the microsporocytes (Cooper, 1952), others only between microsporocytes (Takats, 1959). Mageshvari P. (1954) considers cytomixis a pathological phenomenon, while J. Heslop-Harrison (1966) speaks of his naturality.

A number of researchers believe that the cells, which are chromatin donors, subsequently degenerate (Kamra, 1960; Romanov, 1971), but they can pass meiosis at next stages (Petrov, 1977; Chuvashina *et al*., 1986).

The cytomixis phenomenon is more prevalent in genetically, physiologically and biochemically unbalanced plants such as aneuploids (Gottschalk, 1970), triploids (Salesses, 1970), distant hybrids (de Nettancourt, Grant, 1964) and apomicts (Mantu, Sharma, 1983) than in their diploid relatives.

There are many conflicting opinions and explanations about the cytomixis causes and significance. The recent data indicates that it is a natural phenomenon, which is genetically controlled, under the influence of physiological and environmental factors; besides this anomaly may have some evolutionary significance.

The reason for the transfer of nuclear material at the cytomixis is believed to be the disorder of the actin cytoskeleton, since the transfer of the cell contents through cytomixis canals can be stopped by cytochalasin B, the substance that prevents the growth of actin fibers (Zhang *et al*., 1985). Most researchers agree that this phenomenon must have evolutionary significance (Falistocco *et al*., 1995; Ghanima, Talaat 2003; Boldrini *et al*., 2006), but there is still no agreement about its importance.

In *Houttuynia cordata,* according to J.Z. Gua and colleagues (2012), the cytomixis caused a whole range of cytotypes (2n = 24-128) with x = 8, 9, 12 with a saved genetic gamete heterozygosity and the possibility of additional means of karyotypes phylogenetic development by restoring or increasing the major chromosome row.

Our research has been caused by the dispute on many issues related to the cytomixis phenomenon.

MATERIALS AND METHODS

The biological objects of our research were *Cerasus* Mill plants (species of *Cerasus fruticosa* Pall - Steppe cherry).

Buds taken from natural conditions in the spring were temporally fixed in Carnoy's fixative (3 parts of 96% ethanol: 1 part of glacial acetic acid); the material was stored in 70% ethanol. Microsporogenesis has been studied at temporary squash preparations stained with aceto – hematoxylin according to Topilskaya with co-workers (1975). The material differentiation was carried out in a mixture of 80% chloral hydrate and 45% acetic acid (1: 1).

The microsporogenesis analysis was performed using Leica microscope 2500, and photographic work was performed using digital camera DCM-500 with Scope Photo software. Statistical data was processed in Microsoft Excel environment.

RESULTS AND DISCUSSION

The cytomixis phenomenon was observed by a number of researchers (Soodan, Wafai, 1987; Chmir, 2003; Yandovka, Papihin, 2012 and others) in the earlier microsporogenesis studies of the *Cerasus, Microcerasus* and *Amygdalus* genus. We are the first to describe cytomixis in *C. fruticosa* species.

Analyzing microsporogenesis in *C. fruticosa*, the transfer of chromatin was detected only in 13.0% of anthers under study. A possible reason is that the chromatin migration depends on some external destabilizing factors, most likely - the temperature. It has earlier been established that cytomixis emerge at the gametes formation period does not depend on plants water supply (Yandovka, 2004).

It is known that an increase and to a greater degree a decrease in temperature is of more influence on the microsporogenesis process, up to its complete intermittence (Bennett, Smith, Kemble, 1972; Tarasyuk, 1989). Later, when the destabilizing effect reduces the process continues, but with a significant anomalous phenomena. In this case, the physiological processes failure in the anthers located in peripheral portions of flower buds may

have occurred, due to possible weather anomalies in the premeiotic period.

The cytomixis in the microsporogenesis process of the species under study has shown in two cases: 1) the chromatin transfer from one microspore mother cell into another cell (at different stages of meiosis); 2) the chromatin transfer within the tetrad from one microspore to another.

The cytomixis in microsporocytes has been recorded at different stages of division, its consequences have also been found at different stages.

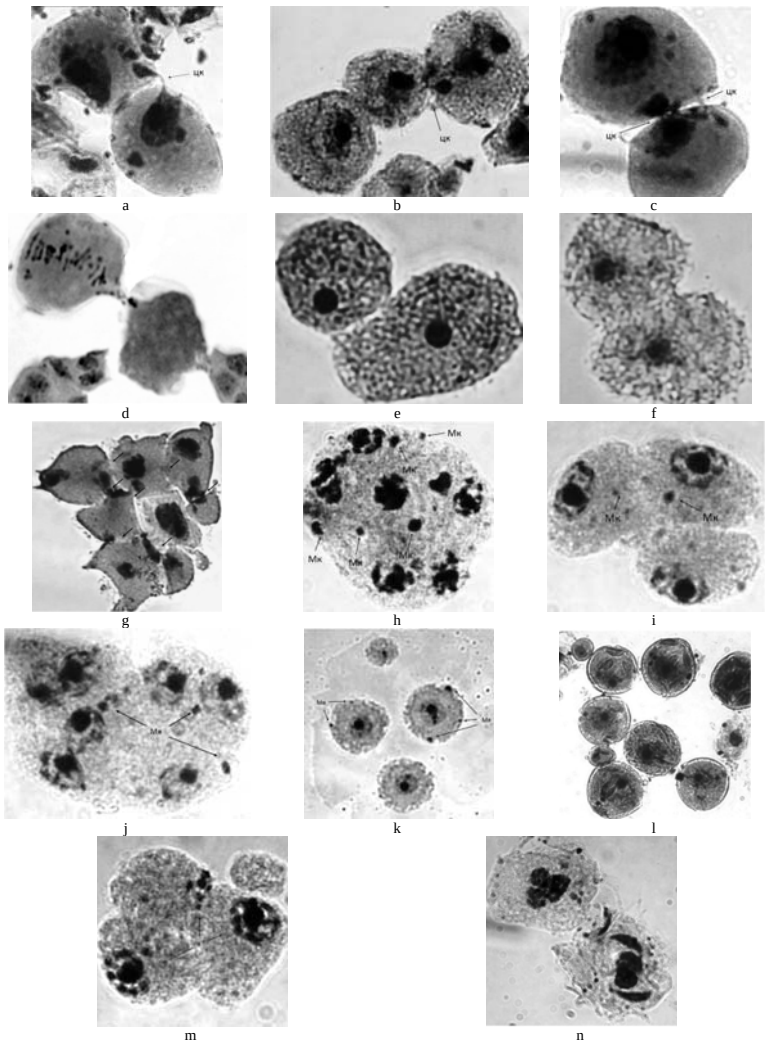

Fig. 1: Cytomixis and its consequences in Cerasus fruticosa: *a-c* - nuclear material transfer from one cell to another in prophase I by one (*a, b*) and two (*c*) cytomixis canals; *d* - cytomixis in metaphase I; *e, f* - the beginning (the formation of three cytoplasmic cords, *e*) and completion (cytoplasm fusion throughout the contact area, *f*) the fusion of two microsporocytes; *g*- multiple cytomixis in a cell group (migration places are indicated by arrows); *h* - hexada with micronuclei; *i* - micronuclei in triad microspores; *j* - heptad with micronuclei; *k* - tetrad of different sizes nuclei with micronuclei; *l* - the pollen of different sizes; *m* - tetrad with microspores without nuclei; *n*- the fusion of tapetum multinucleate cells (Cc - cytoplasmic canal, Mn - - micronucleus; N - nucleus). Magnification: a-h, i, k-n - x 900; h, j - x 1200.

The cytomixis has most been detected at the early stages of chromosome condensation - in prophase I (leptotene, zygotene). In Steppe cherry the number of cells with cytomixis in prophase I in one anther is 9.6 ± 0.5%. In other *Cerasus* species the chromatin transfer is lower at this stage: in Nanking cherry – 2.1 ± 0.9%, in cherry – 8.9 ± 1.0%, in sour cherry – 1.1 ± 1.0%, in Russian almond – 4.6 ± 0.5% (Yandovka, Papihin 2012).

The chromatin transfer from cell to cell at the initial stages of microsporogenesis has earlier been noted by us in other fruit plants of *Malus, Pyrus* genus and their hybrids (Papihin, Yandovka, 2009). The isolated cases of nuclear material transfer have been found at the later stages of microsporogenesis as well: in Sour Cherry (class Vladimir) - in metaphase II, and at the tetrads formation; cherry - anaphase II and telophase II; Russian almond - metaphase I (Yandovka, Papihin 2012).

Chromatin in *C. fruticosa* migrates through plasmodesma (from one to several canals; Fig. 1, *a-e*). However, cytomixis is often a cell fusion (72.5% of cases, Fig. 1 *e, f*). The process begins with the formation of 1-3 cytoplasmic canals between the microsporocytes. Subsequently, the canals enlarge and the cell protoplasts fuse througout the contact area (Fig. 1, *f*). There are two nuclei in the recipient cell.

In Figure 1, *b* the transfer of the nuclear material among the three cells in prophase I and the formation of several micronuclei in a recipient cell is recorded.

The transfer of nuclear material can occur between two or more cells. If the meiocytes make up a dense group, then a multiple cytomixis is possible, in different directions of the recipient cell at that provided these cell walls get in contact (Fig. 1, *g*). The limiting factor of the multiple multidirectional migration of chromatin, in our opinion, is the density of contact between the microsporocytes in the anther.

The future of the additional chromatin that got in microsporocyte by cytomixis may be different. After the transfer in prophase the additional chromatin later is often split up into parts, which at the final stages are disintegrated, and by the end of meiosis they undergo apoptosis. The lysis facts of chromosomes, which dropped out of the division cycle, were proven by Sidorov B.N. and colleagues (1965) in the study of the spindle blocking.

Beginning from the telophase stage of meiotic division, the formation of micronuclei is a cytomixis consequence at earlier stages. Their formation results from improper distribution of chromosomes between the meiocyte daughter cells. Micronuclei (from 1 to 6) have been detected in the microsporocyte cytoplasm (Fig. 1, *i, k*) at the tetrads formation stage. The presence of micronuclei has also been established in the formed pollen grains (Fig. 1, *l*).

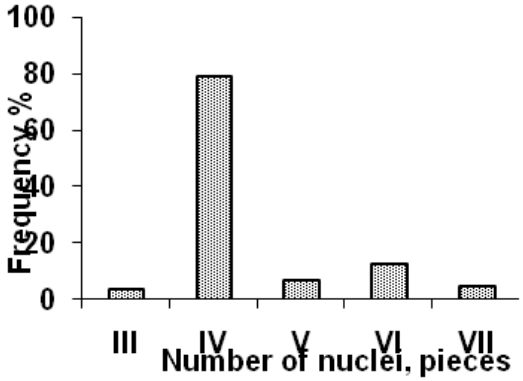

Fig. 2: The formation of the poliad cells at the tetrad formation stage in *Cerasus fruticosa*.

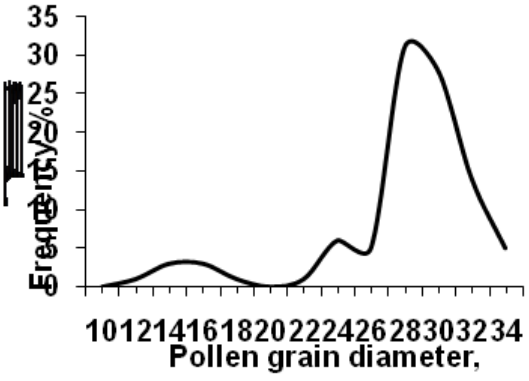

Fig. 3: Pollen sizes variation in *Cerasus fruticosa.*

In some cases, the additional chromatin, which has not been disintegrated till the telophase II and tetrads stages, leads to the formation of cytomixis polyads (Fig. 1, *j)*. Some cells have been detected in telophase II with more than four nuclei - up to 8-9 nuclei and less than four nuclei. As a result, poliads and triads have been found at the forming tetrads stage (Fig. 1, *h-j*, 2).

It has been noted that at a possible cells fusion at the early stages of microsporogenesis most polyads contain micronuclei consisting of several chromosomes and together with the daughter nuclei having a volume close to two nuclei. Polyad nuclei sometimes have an equal volume corresponding to the nuclei of the normally formed tetrads, which indicates that cytomixis and cytokinesis may result without chromosome "losses" at the previous stages. In those cases when a small amount of micronuclei is observed in the cells in the telophase II while the number of nuclei is 5-7, it can be explained by the lysis of individual chromosomes or their small groups at earlier stages by the cytoplasmic enzymes.

At the tetrad formation stage there are some cells with microspores that have no nuclei at all – the effect of the cytomixis that occurred at the early stages of microsporogenesis (Fig. 1, *m*).

Microsporocytes with micronuclei of different sizes form pollen grains of different sizes (Fig. 1, *l*). The analysis of the variation curves of the pollen grains diameter reflects the identified morphological heterogeneity of the formed pollen that emerged from hyper- and hypo-meiocytes that had been the result of violations of the microsporogenesis process at different stages. Figure 3 shows that the curve of the steppe cherry pollen diameter is multimodal.

The curve first peak characterizes the occurrence of cells with a diameter of 10 to 20 ¼m and corresponds to the pollen grains with a small size of nucleus. Such pollen as a result of a genetic imbalance is not functional, i.e. infertile. The next peak is in the range of 24 ¼m. In our opinion, it corresponds to the pollen grains the nuclei of which as a result of cytomixis or some other abnormal phenomena have lost one or more chromosomes, i.e. they are hypoaneuhaploid. This pollen can be functional, but the probability of deviation from the normal process of double fertilization is very high. The size of the pollen bulk (peak3) is 26-34 ¼m. These dimensions correspond to the fertile pollen.

The cytomixis phenomenon at the time of microsporogenesis was detected in tapetum cells. It often occurs with the cells protoplasts fusion throughout the contact area (Fig. 1, *n*).

CONCLUSION

Extrusion of chromatin is one of the distortions of the meiosis processes normal course during the microsporogenesis. The cells with the cytomixis consequences can pass the meiosis stages. Different species have their taxon-specific features of cytomixis during the microsporogenesis.

As a certain amount of nuclear material transfers as a consequence of this phenomenon, it is obvious that the result is the appearance of aneuploid cells. The chromosomes migration causes sporogenic cells sterilization. The cytomixis during microsporogenesis can occur not only between the microsporocytes, but also between tapetum cells.

There are reasons to believe that the cytomixis phenomenon is a natural process that accompanies microsporogenesis, but it has a definite evolutionary significance because of the formation of a certain amount of unreduced pollen as a result.

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