

ORDER OF CHROMOSOME ARRANGEMENT LOCATION IN LATE PROPHASE - EARLY PROMETAPHASE OF MITOSIS IN HAPLOID MAIZE PLANT OBTAINED WITH THE USE OF MUTATION *ig*

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Despite the huge amount of works devoted to the study of mitotic division, there is still a lot of unclear in its mechanisms. For example, insufficient attention has been paid to the processes of cell division in plant forms of different ploidy. Only fragmentary data on haploids in lower plants are available, that makes any generalizations regarding mitosis in haploids of higher plants problematic. Here we report the results of a cytological study of mitotically dividing cells of haploid maize plants. The report demonstrates the effectiveness of the well-known Chase method, based on the use of genetic markers and the *ig* mutation (indeterminate gametophyte) for obtaining and detecting haploids. An effective modification of the simple method of staining cytological preparations with acetocarmine is described. **An essential result obtained is, in our opinion, the detection of a very short moment in the state of chromosomes in a dividing cell of a haploid maize plant, when chromatids have already separated and turned into independent chromosomes, but have not yet begun their movement under the action of kinetochore microtubules.** It is this feature that made it possible to designate this state as late prophase – early prometaphase of mitosis. **An equally important feature of the detected moment is the unusual ordered arrangement of chromosomes, which lie parallel to each other close to each other along their entire length with the centromeres located on one line, which can be considered the equator of the fission spindle.** The revealed fact allows us to assume that an essential role in the formation of such an arrangement of chromosomes is played by the bond of chromosomes with the equator of the nuclear membrane and their subsequent connection with the equator of the fission spindle.



Figure 1. Obtaining patroclinal maize haploids. 1 - tester line T; 2 - hybrid ♀T × ♂ W155; 3 - inbred line W155; 4 – putative patroclinal haploid; 5 - hybrid seed with two embryos.

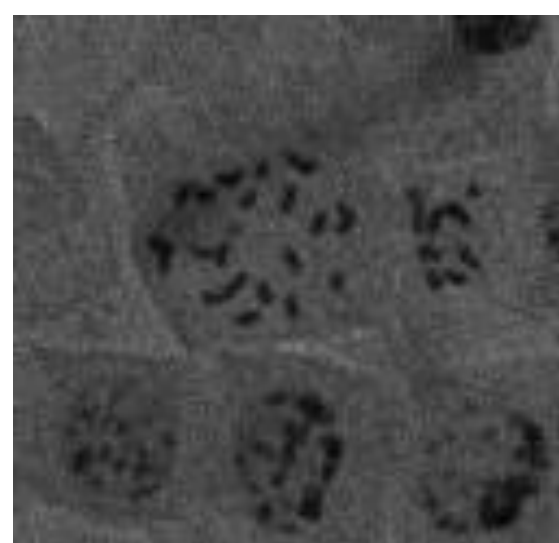


Figure 2. Mixoploidy in the root of a haploid corn plant - the presence of diploid and haploid cells.

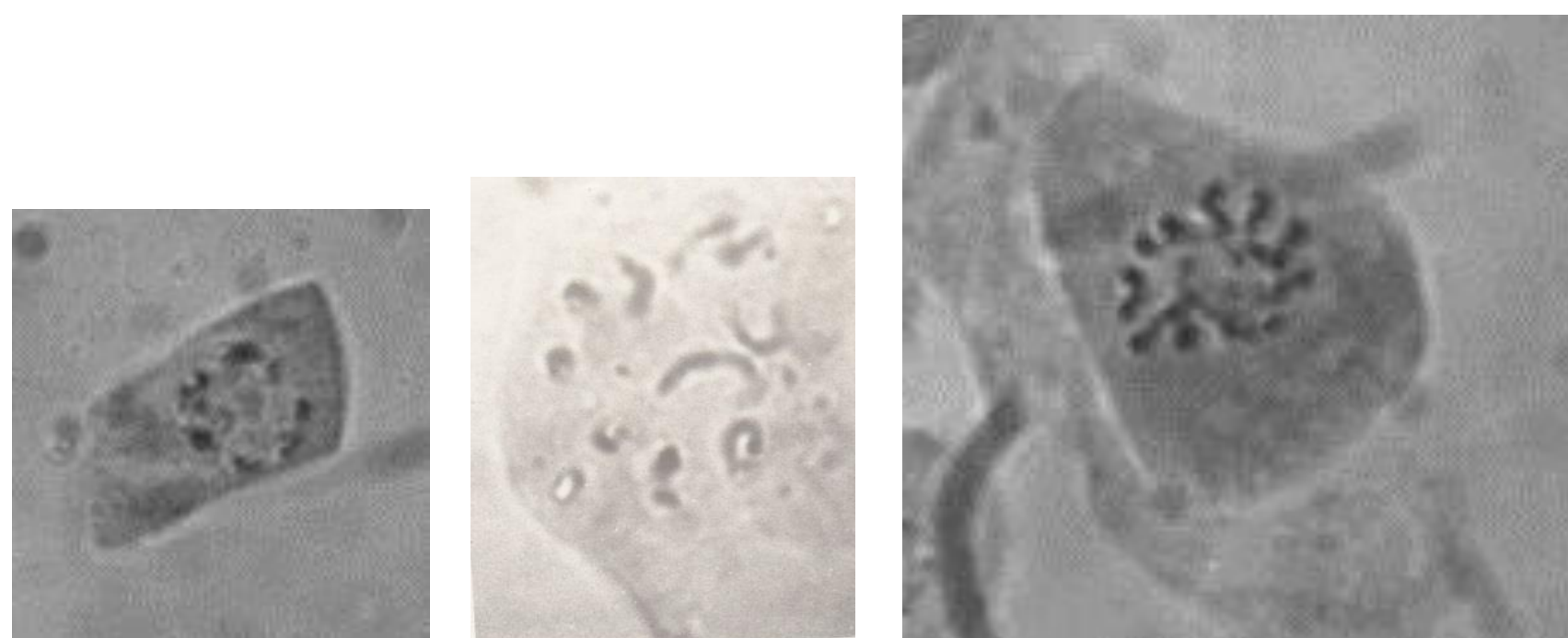


Figure 3. Condensing chromosomes in the prophase of mitosis in the roots of haploid corn seedlings: initial (a), middle (b) and late (c) stages of condensation.



Figure 4. Arrangement of chromosomes in the late prophase - early prometaphase of mitosis at the point of growth of the aerial root of a haploid maize plant.

Staining the aleurone and embryo as marker traits is very effective to search for haploids. Figure 1 shows kernels of maize of the original form, as well as a kernel with a putative haploid. The frequency of detected haploids was about 1: 300, which corresponds to the frequency identified in the work of Kamikl using a test form of the same genotype. In our work, kernels with twin embryos were found (Figure 1). This also matches to the results obtained earlier in the work of Kermikle. Cytological analysis showed that plant cells obtained from crossing ♀T x ♂ W155 and selected for the phenotypic traits of the kernels contain 10 chromosomes (Figures 2–3). These haploids are patroclinal because their chromosomes are derived from the male plant of the W155 line.

In the root tissues of haploid seedlings, mixoploidy may be observed - the presence of contaminating diploid cells along with the main bulk of haploid cells. The image of these cells at the metaphase plate stage is shown in Figure 2.

Attention is drawn to the arrangement of chromosomes in mitotic cells. It can be seen that the condensing chromosomes detected in the seedling roots are located mainly along the periphery of the nucleus (Figure 3). This is in good agreement with the known data on the location of chromatin at the periphery of the nucleus. The haploid cells shown in this figure are in the prophase and differ in the degree of chromatin condensation.

An interesting arrangement of chromosomes was found during the analysis of mitotic division in cells of the aerial roots of a haploid maize plant. The specimen was obtained without applying pressure to the coverslip, which made it possible to largely preserve the native arrangement of chromosomes in the dividing cell (Figure 4). Chromosomes are located closely side by side, parallel to each other, collinearly, forming a kind of ribbon, on the midline of which centromeres are located (Figure 4). Despite the very mild preparation conditions, the chromosome ribbon disintegrated into separate fragments, however, the same pattern is observed in each fragment, which can be interpreted as follows: **the centromeres of the chromosomes are located along the equator of the nucleus or the spindle, and the chromosomes themselves are oriented along the nucleus meridians.** Analyzing the presented image of the preparation, the following can also be noted.

At the stage shown in Figure 4, sister chromatids are already completely separated from each other, i.e. they have become independent chromosomes. They are arranged in the form of a disintegrated ribbon, where the centromeres are on the same line, the position of which can be considered with a high probability as a consequence of the formation of this localization at the equator of the nucleus. Taking this assumption, the arrangement of the separated sister chromatids, characterized as collinear, should be considered as oriented along the meridians of the nucleus (Figure 4). This arrangement is unusual, since in the publications known to us, only the arrangement of centromeres or simply chromosomes along the equator of the fission spindle is noted. The most impressive article devoted to the spatial position of chromosomes in the prometaphase of a mitotically dividing human cell shows the location of centromeres along the equator of the division spindle, but with absolutely free arrangement of the chromosome arms [Magidson et al., 2011]. In a mitotically dividing cell of a haploid maize plant, an even more ordered arrangement of chromosomes was revealed (Figure 4). This phenomenon requires further study.

The unusual location of chromosomes in the late prophase – early prometaphase of mitosis well complements the well-known data on the innumerable variations of all stages of cell division in various tissues in different plant species. All this once again indicates that cell division should not be considered just as a simple distribution of genetic material between daughter cells, but as a stage being specific for each species in the functioning of the genome, during which take place not the simple distribution of genetic material between daughter cells. Specific sequence of functioning of all elements of the mitotic apparatus during mitotic division ensures the maintenance of a specific set of active and inactive genes characteristic for a particular type of dividing cells.