Mitosis and some sunflower genotypes response

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Abstract

Sunflower mitosis ensures consistency of the chromosomes number (the heritable material), and the structural integrity of tissues. The mitosis shall also give an indication of sunflower growth and development and ensure the regeneration of vegetable tissues.

In order to determine the sunflower mitotic activity response, were investigated five sunflower genotypes. They were represented by Barolo, Fleuret, Rigasol, Tuscania and Aldaba hybrids. The mitotic activity analyzed to these genotypes, expressed by mitotic index, was variable from 15.6 % to 21.4 % limits. From this point of view, it was found superiority of the mitotic index percentage to Rigasol hybrid with the 7.21 % bigger than Fleuret hybrid, with the 9.91 % bigger than Tuscania hybrid, with the 22.5 % more than Aldaba hybrid and with the 27.4 % bigger than Barolo genotype.

The determination of mitotic activity to sunflower may contribute revealing the peculiarities of cell division to homozygote and heterozygote genotypes and their correlation with the heterosis.

Key words

mitosis, the mitotic index, sunflower

Mitosis is the process of dividing chromosomes during cell division in eukaryotic cells. Mitosis is followed by cytokinesis, the splitting of the cytoplasm [4]. The function of mitosis is to divide a cell's nucleus with its chromosomes into two daughter cell nuclei, each of which inherits the same number of chromosomes as the parent cell. The intensity of cell division depends on the regulating mechanisms of the cell cycle. In that way the numerical growth on cells in division may be brought about by prolonging the expression of cell cycle machinery. Thus, the increase in the rate of division could be caused by the intensity of transition through cell cycle checkpoints [5].

The intensity of the mitotic activity of a meristematic tissue is determined by the number of cells in division and the intensity of their division. The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are prophase, metaphase, anaphase and telophase. These phases can be distinguished through microscopic analysis. During prophase, the chromosomes condense into shorter and thicker rodlike structures that can be easily seen to consist of two sister chromatids connected by a centromere. At the end of prophase, the nuclear envelope breaks down into vesicles. During metaphase, the chromosomes are fully aligned end to end at the cell's midline at what is known as the metaphase plate. The attachments between sister chromatids to each other split during anaphase, producing single-chromatid chromosomes. During telophase, the nuclear membranes are dephosphorylated and begin to reform around the two sets of chromosomes at either pole, enclosing and separating them from the rest of the cytoplasm [6].

Materials and Methods

First, in order to determine the seed production, the five sunflower hybrids investigated were grown in experimental field of Faculty of Agriculture Craiova. After obtaining the production field, the sunflower seeds were taken to the laboratory for cytogenetic studies.

The sunflower seeds were put for germination in thermostat (24°C - 25°C). Before that they were sprayed with weak solution (0,5%) of K₂MnO₄ in order to avoid any infection. The cytogenetic analysis was made on small roots of 10-15 mm in length. The research material was fixed into a solution of ethanol-acetic acid (3:1) within 24-48 h time; then it was transferred into the 70º ethanol. The fixed roots were softened with 1 N HCl at 60°C for 4 minutes, acid removed by rinsing the tips with water and tips stained with 2% acetocarmine stain for 2 hr.

To carry out the chromosomes view using the optic microscope, these were colorized with the basic fuchsin decolorized solution (Schiff reactive), prepared in the laboratory. The effective colorized operation was the introduction to 3-4 cc colorant solution from sunflower radicles placed in the Petri boxes, at room temperature. After max. 30 minutes, the meristematic tissues were colored in purple-red. To increase the chromosomes-cytoplasm contrast and the

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optimal microscopic preparation view, before the study under the microscope the colored radicules were kept in a 45% acid acetic solution, for 15 minutes, to remove colorant excess [1, 3]. Microscopic slides were done by pressing. From this point of view, the slides were prepared by putting a drop of 45% acetic acid on the root tip, placing the cover slip over the material and tapping with a pencil to disperse the cell.

The chromosomes evidence was realized using the squash method, with the Feulgen-Rossenbeck rapid coloration chromosomes method. The measurements were made under an optic microscope (MBL-2000 type Kruss, Germany), by considering 15 areas per slide. The mitotic index (Im) was calculated by dividing the number of dividing cells by total number of cells multiplied by 100 [3].

After the microscopic preparation study, it was calculated the next index:

1. Mitotic index (Im), or the cells percentage in division to the total number of cells (Im = Nm x 100/Nt). A high mitotic index value shows that the tissue that were in division a large number of cells.

2. The mitotic index phases, or the cells percentage in different mitosis division phases to the total number of cells in mitosis, using these formulas:
   a) Im prophase % = Nprof. x 100/Nm
   b) Im metaphase % = Nmet. x 100/Nm
   c) Im anaphase % = Nanaf. x 100/Nm
   d) Im telophase % = Ntel. x 100/Nm.

Results and Discussion

Although it has been published, in Romania, a monographic sunflower study [7], which presenting comprehensive achievements of sunflower genetics and improvement, however, in this paper we plan update results regarding these species genetics and improvement, in terms of mitosis and phases of mitosis (prophase, metaphase, anaphase and telophase), including the results of this work.

Experimental results concerning the variability of the sunflower mitotic activity are reported in table 1. In this respect, the mitotic activity of the sunflower genotypes studied, expressed by mitotic index, was variable from 15.6 % to 21.4 % limits. From this point of view, the analyze of the cells division highlighted the sunflower hybrids variability and their specificity. Thus, the Rigasol hybrid recorded the highest mitotic activity (21.4 %), followed by the Fleuret (20.6 %) and Tuscania (20.0 %) sunflower hybrids, and the lowest mitotic activity (15.6 %) was found discovered to the Barolo genotype.

The prophase mitotic index (ImP) recorded values included between 48.1 % (Fleuret), 47.5 % (Rigasol), 47.0 % (Tuscania), 45.8 % (Barolo) and 43.2 % (Aldaba). The metaphase mitotic index (ImM) has registered 22.2 % value (Aldaba), 21.3 % (Tuscania), 20.9 % (Rigasol), 20.6 % (Fleuret) and 19.1 % value (Barolo). As regards the anaphase mitotic index (ImA%), its values ranged by 14.7 % (Aldaba), 14.1 % (Fleuret), 14.0 % (Barolo), 13.2 % (Rigasol) and 12.8 % (Tuscania). The telophase mitotic index (ImT%) has registered variable values included between 21.1 % (Barolo), 19.9 % (Aldaba), 18.9 % (Tuscania), 18.4 % (Rigasol) and 17.2 % (Fleuret).

In conclusion, the mitotic activity analyzes as well as the mitotic index phases highlighted the sunflower hybrids genotypic specificity and that the bigger mitotic activity was registered to the sunflower genotype Rigasol. From this point of view, it was found superiority of the mitotic index percentage to Rigasol hybrid with the 7.21 % bigger than Fleuret hybrid, with the 9.91 % bigger than Tuscania hybrid, with the 22.5 % more than Aldaba hybrid and with the 27.4 % bigger than Barolo genotype.

Proportionality intensity cell division to different genotypes is kept constant along the different ontogenetic phases, therefore, the determination of mitotic activity in the sunflower radicules could contribute to cell division particularities revealing to homozygote and heterozygote sunflower genotypes and their correlation with the heterosis. The genotype responses in this study were probably genotype-dependent. The mitotic activity of the sunflower Rigasol genotype pointed out the fact that every single index is characterized by its specificity in accordance with its genotype. The cytogenetic analysis of the genotypes under research showed the distinction in the number of cells to be generally in division and in certain phases of the mitosis, to go in particulars.

Table 1

<table>
<thead>
<tr>
<th>Sunflower genotypes</th>
<th>Nt (X)</th>
<th>Nm (X)</th>
<th>Im (%)</th>
<th>ImP (%)</th>
<th>ImM (%)</th>
<th>ImA (%)</th>
<th>ImT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barolo</td>
<td>32</td>
<td>5</td>
<td>15.6</td>
<td>45.8</td>
<td>19.1</td>
<td>14.0</td>
<td>21.1</td>
</tr>
<tr>
<td>Fleuret</td>
<td>34</td>
<td>7</td>
<td>20.6</td>
<td>48.1</td>
<td>20.6</td>
<td>14.1</td>
<td>17.2</td>
</tr>
<tr>
<td>Rigasol</td>
<td>28</td>
<td>6</td>
<td>21.4</td>
<td>47.5</td>
<td>20.9</td>
<td>13.2</td>
<td>18.4</td>
</tr>
<tr>
<td>Tuscania</td>
<td>30</td>
<td>6</td>
<td>20.0</td>
<td>47.0</td>
<td>21.3</td>
<td>12.8</td>
<td>18.9</td>
</tr>
<tr>
<td>Aldaba</td>
<td>29</td>
<td>5</td>
<td>17.2</td>
<td>43.2</td>
<td>22.2</td>
<td>14.7</td>
<td>19.9</td>
</tr>
</tbody>
</table>
*Notes: The average values of 50 determinations under the microscope; \(N_t\) - the total number of studied cells; \(N_m\) - the total number of cells in mitosis; \(I_m\%\) - the mitotic index; \(I_{mp}\%\) – the prophase mitotic index; \(I_{mm}\%\) - the metaphase mitotic index; \(I_{ma}\%\) - the anaphase mitotic index; \(I_{mt}\%\) - the telophase mitotic index; \(X\) - the average values.

Fig. 1. Illustration of mitotic activity \((I_m\%)\) to some sunflower genotypes

Fig. 2. Graphical representation of the mitosis index phases variability to sunflower \((I_m\%\) Prophase, \(I_m\%\) Metaphase, \(I_m\%\) Anaphase and \(I_m\%\) Telophase)
Conclusions

The mitotic activity analyze as well as the mitotic index phases highlighted variability of sunflower genotypes from this point of view. The mitotic index was varied from 15.6 % to 21.4 % limits. Also, was highlighted genotypic specificity of sunflower hybrids studied and the fact that, the bigger mitotic activity of the meristeme it was recorded in sunflower genotype Rigasol.

The prophase mitotic index (Im P%) recorded values included between 48.1 (Fleuret) % and 43.2 % (Aldaba) limits. The metaphase mitotic index (Im M%) was varied from 22.2 % value (Aldaba) to 19.1 % limits (Barolo). As regards the anaphase mitotic index (Im A%), its values ranged by 14.7 % (Aldaba) to 12.8 % (Tuscania). The telophase mitotic index (Im T%) has registered variable values included between 21.1 % (Barolo) and 17.2 % value (Fleuret).

The results confirm superiority of the Rigasol hybrid in terms of mitotic index percentage. The genotype responses in this study were probably genotype-dependent. In terms of seed production, it was directly correlated with mitotic activity. Thus, the Rigasol hybrid, which recorded the highest mitotic activity, obtained the highest seed production in field.

One knows that most genetic information is concentrated in the nucleus, which is considered the vital center of the cell. The use of photon microscopy methods in cytogenetics allow to show up the peculiarities of the cell cycle. Hence, using cytogenetic methods of analysis is possible to solve partly the problem of heterosis prognosis and of selecting forms or sunflower productive genotypes. So, the determination of mitotic activity to sunflower may contribute revealing the peculiarities of cell division to homozygote and heterozygote genotypes and their correlation with the heterosis. The study of sunflower mitotic activity as well as the mitotic index phases remain an up-to-date problem not only for the purpose of explaining the heterosis nature and mechanism of action on the cellular level, but also for increasing the crops yield.

References