PERIODICO di MINERALOGIA Volume 89, No. 3, 2020

The cytogenetic study of different sunflower's genotypes

ANA CAPATANA
Plant Biology Department, Faculty of Biology and Paedology, Moldova State University, Mateevici 60 street, Chishinau, MD 2019

Abstract

The phenomenon of heterosis is used for the purpose of obtaining rich harvests. This phenomenon represents one of the most important phenomena of the morphogenesis of the plant, it ensures an optimal viability and harvest of the crops. 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.

Keywords: cell division, heterosis, sunflower

Introduction

The study of productivity on F1 plants and their parental forms 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.

The comparative analysis of F_1 hybrids and their parental lines from the point of view of cell division's rate, cell size, photosynthesis rate and the metabolic flux of sugar, proteins and other metabolites makes an essential contribution for revealing the heterosis phenomenon [3]. It was proved that the absolute oil quantity in sunflower's cotyledon cells is relatively constant and doesn't depend on the accumulation of nitrous or other non-lipid substances. The most intensive processes of lipid accumulation take place at the beginning of the period of seed formation, then the processes decrease steadily [7]. The oil quantity at the hybrid organisms level or at the level of more productive forms is determined by a greater number of cells in the whole organism, in the capitulum and the number of seeds in the capitulum, thus it depends on the more intensive mitotic division in comparison with non-productive lines.

Hence, using cytogenetic methods of analysis is possible to solve partly the problem of heterosis prognosis and of selecting forms or productive genotypes.

Materials and Methods

As object for study we have been used the seeds of four Helianthus annuus L. hybrid (Xenia, Oxana, Performer, Valentino) and their parental lines, 2001-2002 year's harvest, offered by The Science & Production Association „MAGROSELECT", in the town of Soroca, Republic of Moldova.

The sunflower seeds were put for germination in thermostat (24°C - 25°C). Before that they were sprayed with weak solution (0,5%) of K_2MnO_4 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. In order to visualize the chromosomes, they were colored with carmine-acetic solution [4]. Microscopic slides were done by pressing.

The ***mitotic activity (MA)*** and ***the indexes of the mitotic phases (I_f)*** were calculated according to the following formulae [4]: $MA = \frac{N_M \times 100\,\%}{N_t}$, where: N_M – is the total number of cells in mitosis, N_t - is the total number of cells under investigation; $I_f = \frac{N_f \times 100\%}{N_M}$, where: N_f - is the total number of cells which in a certain phase, N_M - is the total number of cells in mitosis.

The cytogenetic results were statistically analyzed according to the following formulae [4]: $m = \sqrt{\frac{P(100-P)}{n}}$, where: ***m*** - standard error, ***P*** - mean value shown in %, ***n*** - the total number of cells under investigation; $td = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}$, under: ***td***- reliability of data criterion, ***M*** – mean value, ***m*** - standard error.

Results and Discussion

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 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 [1].

The intensity of cell division per unit length may present physiological importance [2]; for instance, the speed of cell division might reflect the abundance of regulatory factors with an activity proportional to its concentration. It is considered that cell division is measured on the basis of the cell number, because the new cell results only from the division of a parent cell.

It was proved that the average cell cycle duration for Arabidopsis thaliana was constant for the period of 6-10 days starting with the germination [1]. Thus, it is possible to presume that the proportionality of the intensity of cell division of different genotypes is maintained constant in different ontogenetic phases of plant development.

Mitotic activity of the first generation hybrids (F_1). The analysis of the obtained results has pointed out the differences that take place at the mitotic activity (MA) level of hybrid genotypes, which were classified in decreasing order according to the value of this index /Table 1; Diagram1/: Oxana, Performer, Xenia, Valentino.

Table 1. Mitotic activity and mitosis phase indexes of 1st generation hybrids

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
Performer	9128	1054	11,5±0,3	55,8	21,1	11,6	13,8
Valentino	8876	838	9,4±0,3	47,4	24,3	15,7	12,4
Xenia	8485	930	10,9±0,2	42,3	22,1	21,6	13,8
Oxana	9263	1143	12,3±0,4	47,6	20,9	13,3	17,9

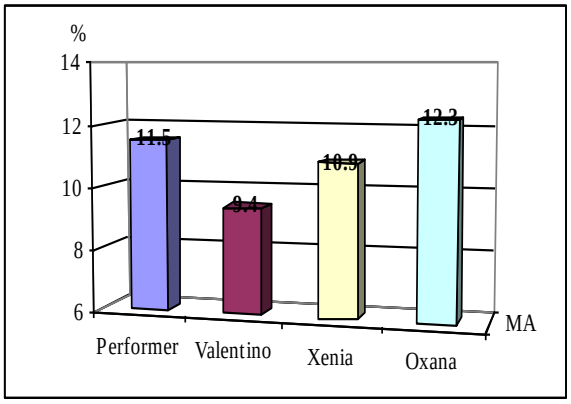

Diagram 1. Study of mitotic activity in F_1

Studying the mitotic activity and mitosis phase indexes, it is observed that most of the MA is found in the hybrid Oxana MA -l2,3%, and the least MA is found in the hybrid Valentino-9,4%.

The comparative analysis of MA has shown the following differences: the hybrid Oxana has appeared to have 30,85% more intensive MA as compared to Valentino, for the Performer hybrid the intensity of meristematic cells division was equal to 22,34%, and for the Xenia the MA index was 15,95% higher than that of the same hybrid.

Mitotic activity in paternal genotypes. The research of mitotic activity in paternal genotypes did not show any essential differences. The studied genotypes can be arranged in a decreasing order according to the manifested value of the MA /Table 2 & Diagram 2/: Oxana /Performer, Xenia, Valentino.

Table 2. Mitotic activity and mitosis phase indexes in paternal genotypes

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
Performer (Oxana)	7609	453	11,2±0,5	47,3	27,2	15,6	9,8
Valentino	9220	849	9,2±0,4	47,3	24,9	16,7	10,9
Xenia	9137	932	10,2±0,3	45,9	27,1	15,8	11,0

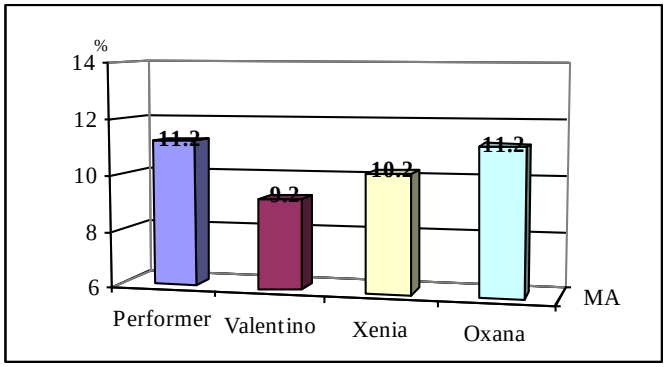

Diagram 2. Mitotic activity indexes in paternal genotypes

Analyzing the MA indexes & the mitosis phase indexes, one can notice the following thing; namely, that the minimum value corresponds to the paternal line Valentino MA -9,2%, while the maximum value corresponds to the Oxana /Performer paternal genotypes-11,2%.

The comparative study of the MA shows the following differences: the paternal genotype Oxana/Performer is 21,73% more active than the Valentino genotype, and the paternal genotype Xenia is characterized by the intensity division of meristematic cells with 10,86% more active in comparison with the same genotype.

Mitotic activity in maternal genotypes. The analysis of the results has emphasized some differences at the MA level of the maternal lines. The studied maternal genotypes can be classified into the following decreasing order from the point of view of mitotic activity /Table 3 & Diagram 3/: Oxana, Performer, Xenia, Valentino.

Table 3. Mitotic activity and mitosis phase indexes in maternal genotypes

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
Performer	8020	858	10,6±0,4	46,5	23,4	17,3	12,7
Valentino	8228	683	8,3±0,3	43,9	22,4	17,5	16,1
Xenia	8906	820	9,2±0,4	48,0	25,2	14,6	10,3
Oxana	6298	712	11,3±0,3	31,6	20,6	11,6	10,2

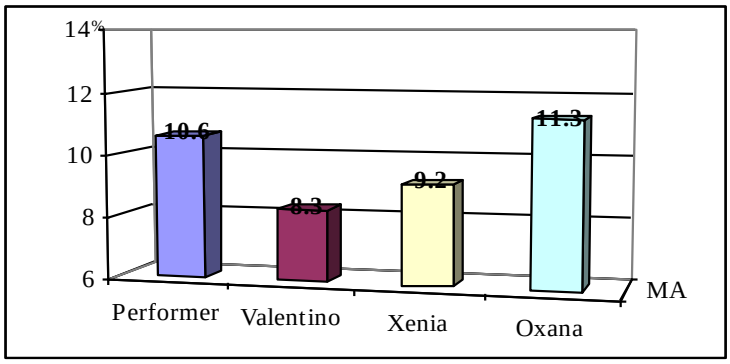

Diagram 3. Mitotic activity indexes in maternal genotypes

The comparison of the MA indexes made it possible to state that this index has shown the least possible value of 8,3% with the Valentino genotype, while the biggest rates of cell production of 11,3% were revealed in the Oxana genotypes.

As compared to the Valentino genotype which showed the least values, the other genotypes evinced a 10,84% higher MA value for the Xenia genotype, 27,71% for the Performer genotype and 36,14% for the Oxana genotype.

Study of mitotic activity within the framework of analyzed "families". With the help of cytogenetic investigations we followed up: the number of cells in different stages and the mitotic activity indexes (for the "families" Performer, Valentino, Xenia and Oxana.

The Performer "family". The obtained results in the analysis of microscopic slides were useful for calculating the following mitotic indexes: mitotic activity (MA), prophase index (P), metaphase index (M), anaphase (A) and telophase index (T). The value of mitotic indexes are shown in Table 4 and presented in Diagram 4.

Table 4. Mitotic activity and mitosis phase indexes in the Performer "family"

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
genotype ♂	8020	858	11,2±0,5	47,3	27,2	15,6	9,8
genotype ♀	8569	960	10,6±0,4	46,5	23,4	17,3	12,7
F_1	9128	1054	11,5±0,3	55,8	21,1	11,6	13,8

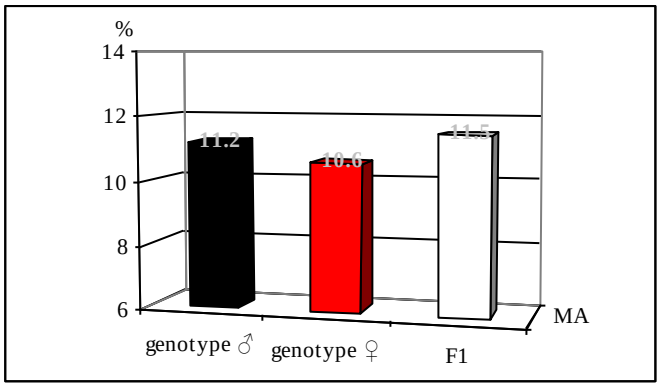

Diagram 4. Mitotic activity indexes for the Performer "family"

The analysis of the MA pointed out the peculiarity of every index for different genotypes of sunflower. The cytogenetic research of the studied genotypes showed the differences in the number of cells in the division stage in general and in certain phases of the mitosis in particular.

These results revealed that the most intensive division of the meristematic cells was ascertained for the 1st generation hybrid, and the lowest activity was ascertained for the maternal genotype, although the differences are not so significant. Thus, it was stated that already at this stage the Performer hybrid exceeds the parental lines from the point of view of mitotic activity, namely in comparison to the maternal genotype it is 8,49% more active and 2,67% as compared to the paternal line /Diagram 4/.

The Valentino "family". The Valentino „family" genotypes were studied in the same way. The data obtained from the analysis of microscopic slides made it possible to calculate the mitotic indexes as well as the mitosis phase indexes, which are presented in Table 5 and Diagram 5.

Table 5. Mitotic activity and mitosis phase indexes in the Valentino "family"

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
genotype♂	8228	683	9,2±0,4	47,3	24,9	16,7	10,9
genotype ♀	9220	849	8,3±0,3	43,9	22,4	17,5	16,1
F_1	8876	838	9,4±0,3	47,4	24,3	15,7	12,4

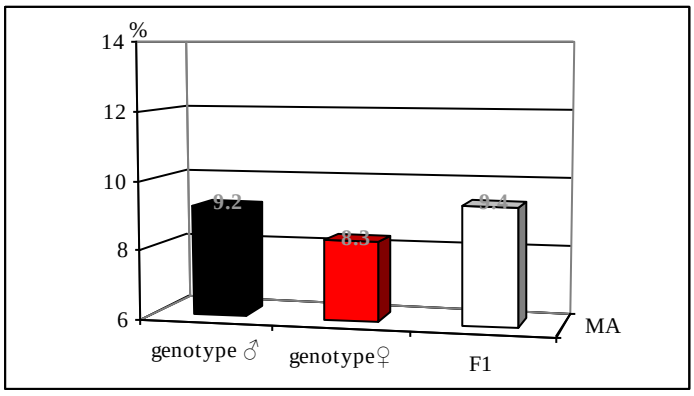

Diagram 5. Mitotic activity indexes for the Valentino "family"

The results obtained show that the mitotic activity was more intense in the hybrid root tip and the lowest value was ascertained in the maternal genotype. It is pointed out that similarly to the Performer family, the Valentino hybrid exceeds both parental lines already at the earliest stages of its development in its mitotic activity. As for the maternal line, the MA is 13,25% more intensive and 2,17% more proactive as compared to the paternal line /Diagram 5/.

The Xenia "family". The similar to the above mentioned research was carried out for the Xenia "family". The results obtained served for calculating the mitotic indexes. The index values are presented in Table 6 and Diagram 6.

Table 6. Mitotic activity and mitosis phase indexes in the Xenia "family"

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
genotype ♂	8906	820	10,2±0,3	45,9	27,1	15,8	11,0
genotype ♀	9137	932	9,2±0,4	48,0	25,2	14,6	10,3
F_1	8485	930	10,9±0,2	42,3	22,1	21,6	13,8

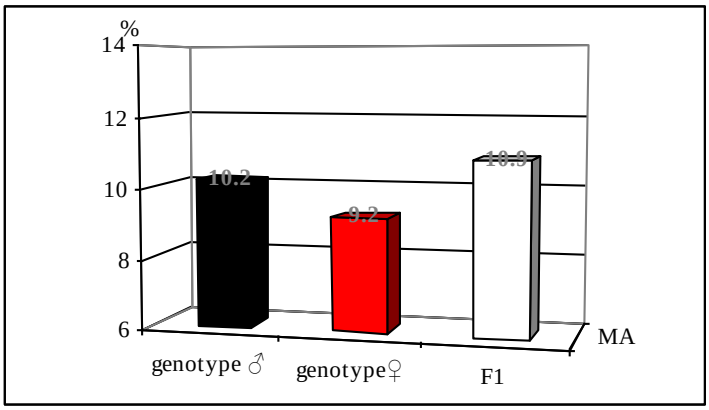

Diagram 6. Mitotic activity indexes for the Xenia "family"

It was noted that the mitotic activity was more intensive in the meristematic tissue in hybrid root apex and the most reduced intensity was ascertained for the maternal genotype. It was found that similarly to the other "families" studied, the same phenomenon occurs: from the early stages of its development the Xenia hybrid is characterized by an intensive growth, exceeding both parental lines. Thus, the cell division is 18,47% more proactive for the maternal genotype and 6,86% as compared to that of the paternal one /Diagram 6/.

The Oxana "family". A cytogenetic study was realized on the Oxana "family" genotypes. The results obtained in the analysis of the microscopic slides were used to count the mitotic indexes. The values of the mitotic indexes are presented in Table 7 and Diagram7.

Table 7. Mitotic activity and mitosis phase indexes in the Oxana "family"

Variants	Studied cells	Cells in mitosis		Phase indexes, %			
		N	M ± m, %	P	M	A	T
genotype ♂	6298	712	11,2±0,5	47,3	27,2	15,6	9,8
genotype ♀	8569	960	11,3±0,3	31,6	20,6	11,6	10,2
F_1	9263	1143	12,3±0,4	47,6	20,9	13,3	17,9

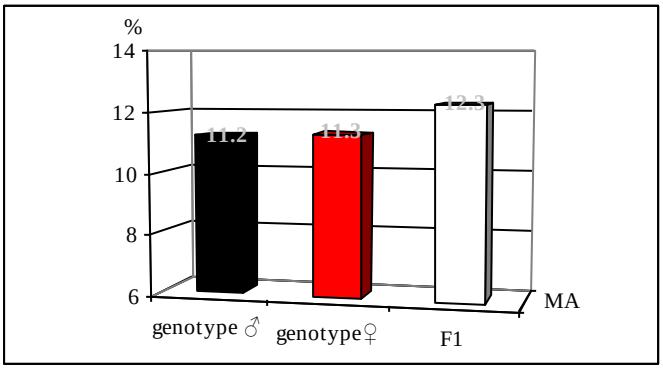

Diagram 7. Mitotic activity indexes for the Oxana "family"

The mitotic activity of the Oxana "family" 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.

The analysis of the results confirms the fact that the top activity of the meristems was noted in F1, while the lowest was recorded in the paternal line with slight differences. It was noticed that already for the period of 1st 24 hours after germination the Oxana hybrid exceeds in point of development the paternal genotypes. It is 9,82% more active as compared to the parental genotype and 8,84% more intensive in comparison with the maternal genotype /Diagram 7/.

Conclusion

To sum up the results, we can establish the following: the study of mitotic activity has shown that the hybrid genotypes irrespective of the studied family the highest intensity of cell division was recorded for the Oxana "family" genotypes, followed by the Performer, the Xenia and the Valentino "family" genotypes. The mitotic activity of paternal genotypes has proved to be more active than that of maternal genotypes in three of four cases.

The superiority of 1st generation hybrids can be already observed at an early stage. The analysis of the mitotic activity and the mitotic phase indexes in hybrid genotypes is higher in all four cases as compared to the paternal and the maternal genotypes.

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