THE EFFECT OF SOME CHEMICAL MUTAGENS EFFECT ON THE MITOTIC DIVISION TO *DIGITALIS LANATA* EHRH

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Abstract

The little woolly finger seeds (*Digitalis lanata Ehrh*.) Lanata 1 variety, were treated with three chemicals, each of these with four concentrations: 2,4-D acid, ethidium bromide and colchicine, in four concentrations each (0.01%, 0.02%, 0.03%, 0.04%), at every experience the results were compared with the control (untreated).
The mutagenic chemicals in the mentioned concentrations influenced the mitotic cycle from the root meristems, by modifying the mitotic index and through changes of some of its phases, by the occurrence of some changed phases, namely some chromosomes reorganization (single or multiple chromosomal bridges, residuary chromosomes, chromosomes fragments and micronuclei).
The research has established the correlations resulted from the interactions of the biological material and the mutagen (the biological material sensitivity) and between the substances concentration and the frequency of the chromosomal aberrations.

Key words: chemical mutagens (2,4-D acid, ethidium bromide, colchicine), ana-telophase aberrations

MATERIAL AND METHOD

The biological material was represented by the little woolly finger variety, Lanata 1, created by F. Silva (1969) at SCPMA Fundulea. The variety was approved in 1974 and registered once again in 1999.

The Lanata 1. variety is characterized by a fairly pronounced polymorphism in a morpho-physiological aspect, with an average production of 1600 kg / ha of dry leaves and an average content of 0.21% lanatozid C, exceeding the average content by nearly 55% in this active principle, the existing local populations.

The dry seeds of the Lanata. l variety were treated with three chemical mutagens (2,4-D acid, ethidium bromide and colchicine), each in four concentrations (0.01%, 0.02%, 0.03%, 0.04%), with two treatment periods, 3 hours and 6 hours along the control (seeds germinated in distilled water). The treatments were carried out in Petri dishes, after which, the seeds were rinsed with distilled water and passed in Petri dishes on moistened filter paper for germination.

Each mutagen and treatment variant was performed in three repetitions to capitalize on the experimental results, by the variation analysis.

For the cytogenetic investigations were harvested little roots with length of 1.0 - 1.5 cm, for the temporary and permanent microscopic preparations, following the classic work protocols: prefixing, fixation, hydrolysis, staining and inclusion in Canada balsam (Ţîrdea Gh, Leonte C., 2003, Butnaru, Gallia et al., 2004).

The microscopic preparations were examined at the Hund-Wetzlar photon microscopes, the photos were made at the 100x (dip) objective, using a Cannon digital camera, adapted to the microscope.

The mitotic index was calculated using the following formula:

$$I_m(\%) = \frac{N_m * 100}{N_t}$$, where:

N_m – total number of cells in division;
N_t – total number of examined cells.

Of the total number of cells in mitotic division, for each variant (substance, concentration), it has been determined the frequency of the cells with ana-telophase chromosomal aberrations, which eventually were statistically interpreted using the variation analysis.

RESULTS AND DISCUSSIONS

The mitotic activity of a meristematic tissue or other types of tissue is estimated by the mitotic index (I_m), which involves determining the number of cells in division, in one of the mitosis phases, of the total number of analyzed cells. This general index can be also partially analyzed for each phase of the analysis: prophase (I_p), metaphase (I_M), anaphase (I_A) and telophase (I_T) (Ţîrdea Gh, Leonte C., 2003).

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Considering that the mitosis, as type of division of the somatic cells, in addition to the role of maintaining a constant diploid number of chromosomes, provides the tissues and organs growth, we are interested how a series of internal and external factors acts on this process.

In the case of the experimental mutagenesis, the mitotic index is a microscopic feature that provides precise information on the action's way and efficiency of some physical, chemical or biological mutagens.

Following the work protocol described above, it has been determined the mean values percentages of mitotic index (I_m%) from our experiments presented in (table 1).

The mitotic index in the control variants had similar mean values between 15.89% and 16.42%, these small variations are explained by the variations of those three factors that we've tried to maintain them constant: humidity, temperature and light.

In the control variants, the mitotic index mean value fluctuated in the lower limits, between 15.89% (colchicine) and 16.42%, (2,4-D acid), the differences were due to the small variations of the humidity and temperature from thermostat.

The minimum concentration of treatment, 0.01% of 2,4-D acid, ethidium bromide and colchicine had a slight stimulating effect of the mitotic index, with mean values between 16.83% (ethidium bromide) and 17.36% (colchicine), but the differences compared to the control were not statistically assured.

Starting with the 0.02% concentration for the first three substances, the mean values of the mitotic index are decreasing but statistically assured differences were measured only at the last concentrations, 0.03% and 0.04%. At the maximum concentration of treatment, 0.04% of those three chemicals, the mitotic index had mean values between 3.18% (ethidium bromide) and 4.69% (2.4-D acid). The differences compared to the control, on all three chemicals, were significantly distinct and in the ethidium bromide's case were very significant.

Table 1

The effect of the mutagenic chemicals treatment on the mitotic index (%) on Digitalis lanata Ehrh., Lanata L. variety

Substanţa (agentul mutagen)	Concentraţia (%)				
Acidul 2,4-D	martor	0,01	0,02	0,03	0,04
	16,42	17,06	12,36	$8,22^{0}$	$4,69^{00}$
Bromura de etidium	martor	0,01	0,02	0,03	0,04
	16,30	16,83	10,24	$6,31^{00}$	$3,18^{000}$
Colchicină	martor	0,01	0,02	0,03	0,04
	15,89	17,36	15,26	$9,21^{0}$	$4,18^{00}$
DL 5%=6,38	DL 1%=8,92		DL 0,1%=12,54		

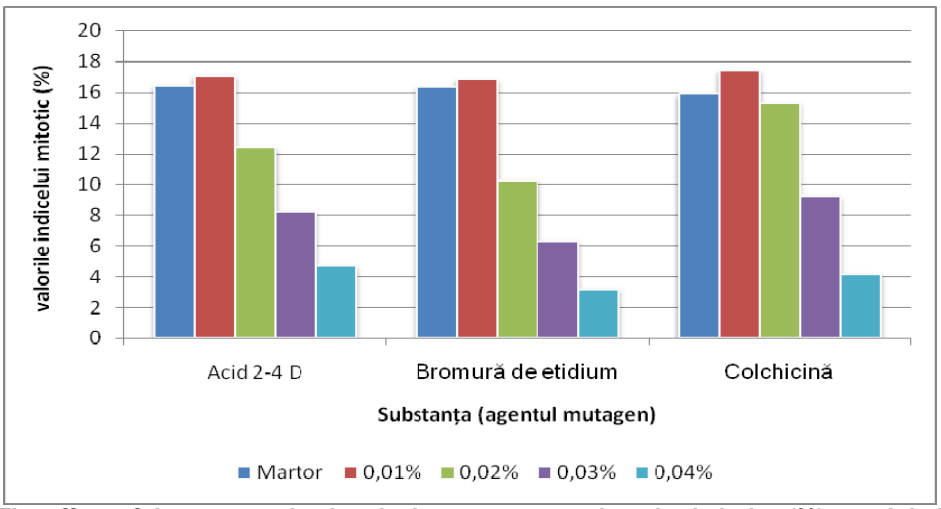

Figure 1 **The effect of the mutagenic chemicals treatment on the mitotic index (%) on *Digitalis lanata Ehrh., Lanata* L. Variety**

Analyzing the mutagenic effect of those three chemicals, reflected by the mitotic index, we can appreciate that the ethidium bromide had the most pronounced effect (3.18%), followed by colchicine (4.18%) and the 2-4 acid D (4.69%).

The ana-telophase aberrations frequency in mitosis

The frequency of the chromosomal aberrations in mitosis is one of the most important early indicators regarding the mutagenic action of all mutagens (physical, chemical or biological). (Nicolae, I, 1978)

After the treatment with those three chemical mutagens in mitosis, it has been identified the following types of chromosomal aberrations:

- Interphases with micronuclei;
- Metaphases with chromosomal fragments and delayed chromosomes;
- Anafazes with chromosomal fragments, delayed chromosomes and chromosomal bridges;
- Telophases with micronuclei.

Many researches of experimental mutagenesis have shown that, of the total cells with chromosomal aberrations, the highest frequency of the chromosomal restructuring has differentiated in the mitosis and meiosis anafaza and telophase (Nicolae I., 1978). This observation had promt applicability in mutagenesis research, the mutagenic effect of mutagens, was considered more expeditiously using the frequency of ana-telophase aberrations in cell division. (Vatavu, Roxana, 2008).

Following the work protocol described above, it has been analyzed the frequency of the ana-telophase aberration in mitosis of the little finger's variety, Lanata l., the results were presented in (table 2).

Within the control variants, the frequency of the ana-telophase natural aberration had very low values from 0.00% to 0.42%, values that correspond to the literature data. (Hartl, DL, Freifelder, D., Snyder, LA, 1988)

After the treatments performed with 2.4-D acid, the frequency of the aberrant ana-telophases ranged between 6.25% to 0.01% concentration and 21.61% to the maximum value of treatment 0.04%. The maximum concentration of 2.4-D acid, 0.03 and 0.04%, the differences compared to the control were significantly distinct.

Table 2

The frequency of ana-telophase mitotic aberration (%) induced by chemical mutagens to *Digitalis lanata Ehrh*. Lanata L. variety

Substanţa (agentul mutagen)	Concentraţia (%)				
Acidul 2,4-D	Martor	0,01	0,02	0,03	0,04
	0,00	6,25	9,32	16,20**	21,61**
Bromura de etidium	Martor	0,01	0,02	0,03	0,04
	0,08	10,24	15,04**	19,34**	27,34***
Colchicină	Martor	0,01	0,02	0,03	0,04
	0,42	9,32	14.20*	18,64**	21,44**
DL 5%=10,61 DL 1%=14,32 DL 0,1%=22,10					

The ethidium bromide had a much stronger mutagenic effect, the frequency of the ana-telophase aberration recorded values between 10.24% at the minimum concentration (0.01%) and 27.34% at the maximum concentration (0.04%), the differences compared to the control were statistically assured.

The treatments performed with different concentrations of colchicine had a pronounced effect on mitotic division, the ana-telofaphases frequency recorded values between 9.32% to the minimum concentration of 0.01% and 21.44% at the maximum treatment concentration of 0.04%.

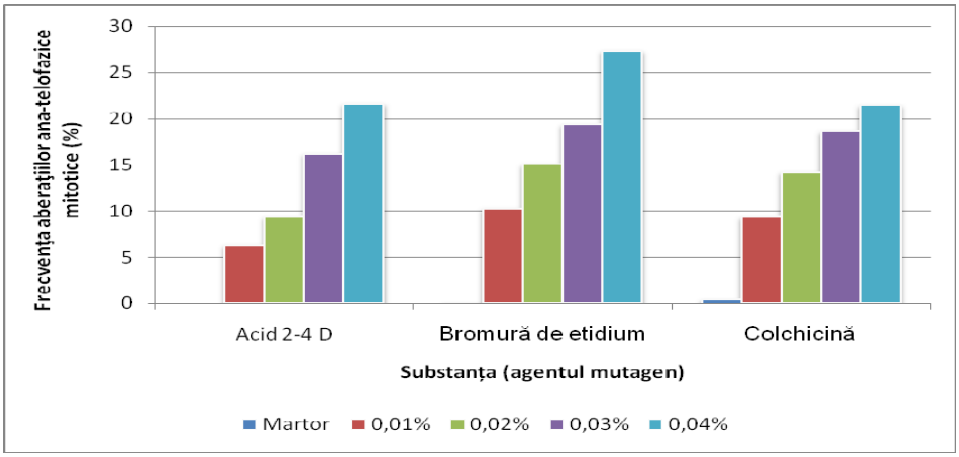

Figure 2 **The frequency of ana-telophase mitotic aberration (%) induced by chemical mutagens to *Digitalis lanata Ehrh*. Lanata L. variety**

Globally analyzing the mutagenic effect of those three chemicals, in terms of frequency of the ana-telophase aberration in mitosis, it can be observed that the ethidium bromide has the strongest effect, followed by the treatment with colchicine and 2.4-D acid.

In the analyzed table, it has been observed that naturally can appear in a small percentage chromosomal aberrations but they, usually, can't manifest phenotypically because they are dimmed by the normal cells.

In figure 3 to 10 are presented the most important chromosomal aberrations that have occurred in Digitalis lanata plants under the influence of the chemical mutagens: colchicine, 2-4 D acid and ethidium bromide.

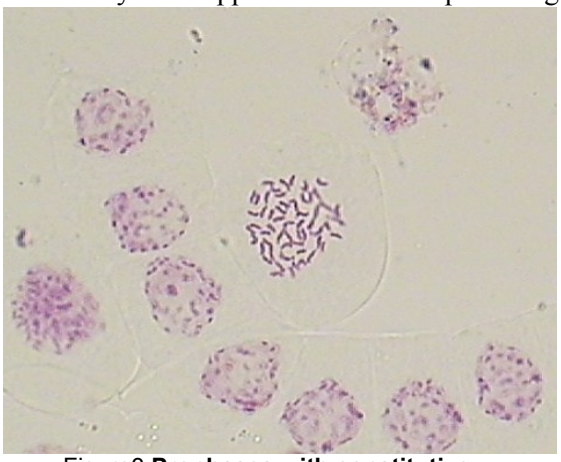

Figure3 **Prophases with constitutive heterochromatin and normal metaphase**

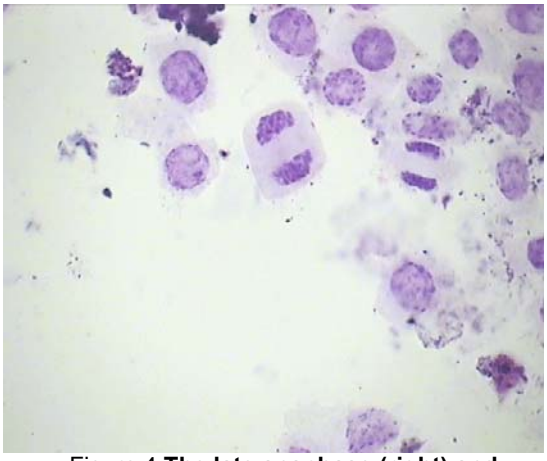

Figure 4 **The late anaphase (right) and the early telophase (left) - normal**

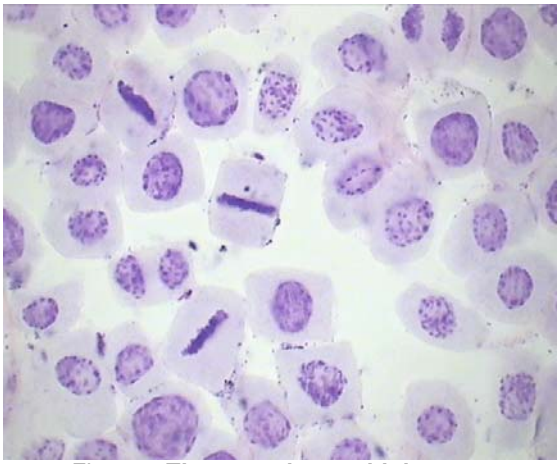

Figure 5 **The metaphase with late chromosomes**

Figure 6 **The early anaphase with late chromosomes**

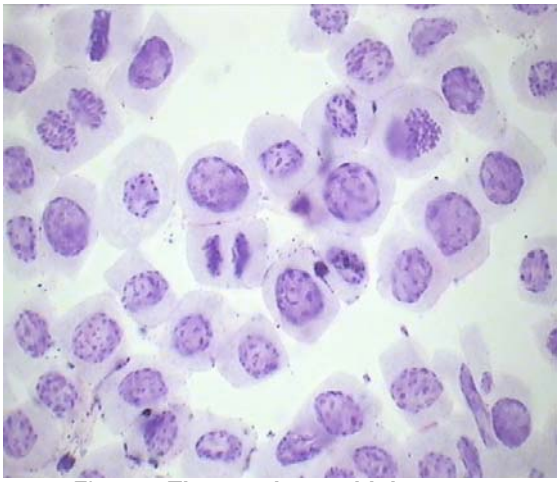

Figure 7 **The anaphase with late chromosom (middle) and thetelophase with micronucleus (low).**

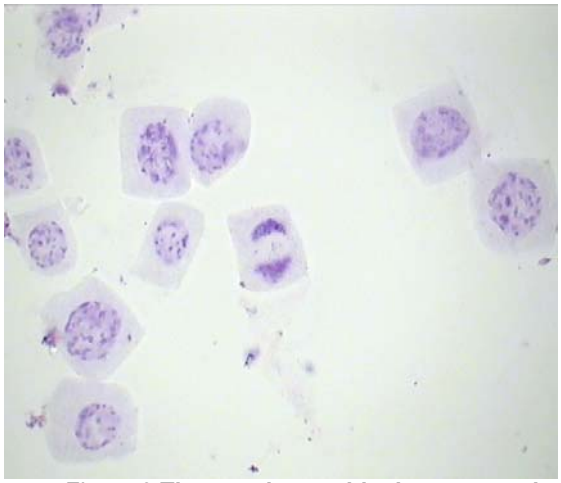

Figure 8 **The anaphase with chromosomal fragments**

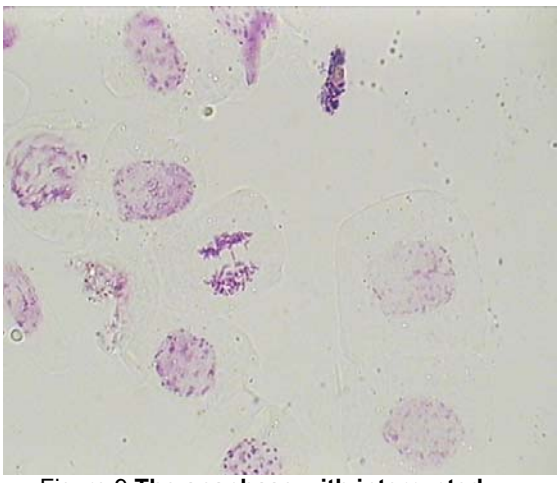

Figure 9 **The anaphase with interrupted bridge 1**

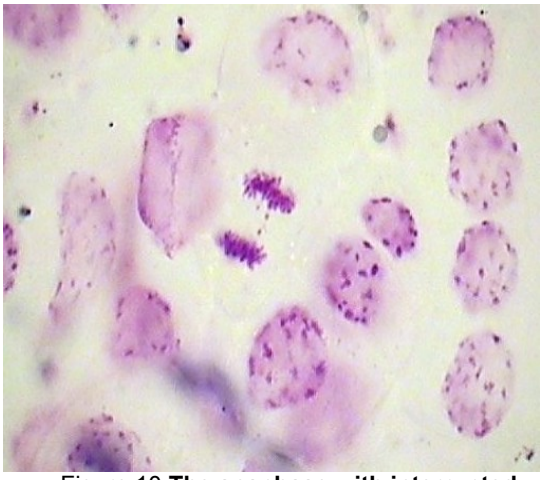

Figure 10 **The anaphase with interrupted bridge 2**

CONCLUSIONS

After this research we have made the following conclusions.

Analyzing the mutagenic effect of the three chemicals, reflected by the mitotic index, we can appreciate that the ethidium bromide has the most pronounced effect, reducing the mitotic index up to 3.18%, followed by colchicine (4.18%) and 2.4-D acid (4.69%).

The low concentrations (0.01%) of the three chemicals, had a slight stimulating effect of the mitotic index, with mean values between 16.83% (the ethidium bromide) and 17.36% (the colchicine), the differences compared to the control were not statistically assured.

Analyzing the frequency of the ana-telophase aberration in mitosis, it has been found that the ethidium bromide had a pronounced mutagenic effect, causing the occurrence of 27.34% chromosomal restructuring, being followed by colchicine and 2.4-D acid.

In the case of the treatments performed with the three chemicals, it has been found the existence of positive correlations between the concentration we have used and the frequency of the ana-telophase aberration.

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