CYTOGENETIC EFFECTS INDUCED BY TREATMENT WITH ETHIDIUM BROMIDE AT *PAPAVER SOMNIFERUM* SPECIES

EFECTE CITOGENETICE INDUSE DE TRATAMENTUL CU BROMURĂ DE ETIDIUM LA SPECIA *PAPAVER SOMNIFERUM* L.

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Abstract: *Our study shows some changes that are at Papaver somniferum, at the division cells from the radicular apex, as the results of the treatment used with ethidium bromide. One noticed a decrease of the mitotic index at the variants analysed. The percentage of ana-telophases with aberration decreased slightly at the variants treated with mutagen substance. The treatment with ethidium bromide in the concentration 0,01%, 0,02%, 0,03% and 0,04% for 6 hours of on opium poppy radicular meristems were expressed by chromosomial mutations, especially in ana-telophases: bridge, thick bridge, multiples bridges, chromatic material expulsed and fragment, retarded and expulsed chromosomes, interphases with micronuclei. Mutations are changes of the genetic, physiologic and biochemical equilibrium of the some species; they influence negatively its efficiency. Mutants can have however, important features that can be used in the improvement programmes of that species (Ghiorghiță, I. G., 1999).*

Rezumat: *Studiul nostru are în vedere evidențierea modificărilor din diviziunea celulară din apexul radicular de la Papaver somniferum, ca rezultat al tratamentelor cu bromură de etidium. Am observat o scădere a indicelui mitotic la variantele analizate. Procentajul ana-telofazelor cu aberații a scăzut uşor la variantele tratate cu substanțe mutagene. Tratamentul cu bromură de etidium în concentrații de 0,01%, 0,02%, 0,03% şi 0,04% pentru 6 ore a meristemelor radiculare de mac s-a exprimat prin mutații cromozomiale, în special în ana-telofaze: punți, punți îngroşate, punți multiple, material cromatic expulzat şi cromozomi expulzați, interfaze cu micronuclei. Mutațiile reprezintă modificări genetice, fiziologice şi biochimice asupra echilibrului speciilor; ele influențează negativ eficiența lor. Mutantele pot fi importante pentru îmbunătățirea programelor acestor specii (Ghiorghiță, I. G., 1999).*

MATERIAL AND METHOD

Biological material 2 years seeds belonging to the species of Papaver somniferum L, variety De Botosani from the Agricultural and Zootechnic Researches Station of Secuieni, Neamt, harvest 2005. The mutagen factor used was represented by ethidium bromide in concentration of 0.01%, 0.02%, 0.03% and 0.04%, which represents the variants used in the experiment. Each variant of treatment sue on the seeds for 6 hours.

The germination was secured in Petri plates, on filter paper, at room's temperature (24ºC ± 2ºC). The harvest of the roots was realised when they were 10-15 mm high and they had been fixed in absolute ethylic alcohol / glacial acid acetic

(3:1), at room's temperature for 10-20 hours. To keep this biologic material for analyses it was secured in ethylic alcohol 70%, in the refrigerator. It was used a solution of HCl 50%, for hydrolysis, for 14 minutes and to stain at was used a Carr reagent. The preparations were realised with the squash method, they were seen at the microscope with 20X object lens and they were photographed with a 100X objective in immersion.

RESULTS AND DISCUSSIONS

a) **The mitotic index (MI)**

The treatment with ethidium bromide determined a decrease of the value of the mitotic index at all the variants. The most important decrease was recorded at the variant 0.04% (0.13), followed by the variant 0.03% (0.15). The concentrations applied had an inhibitory effect on the process of mitotic division at the cells level of the radicular apex (tab. 1, fig. 1).

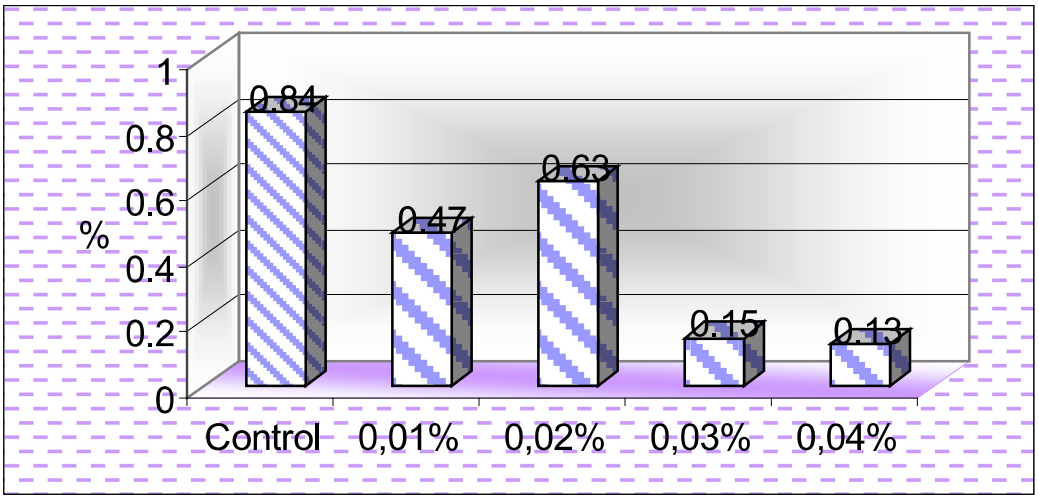

Fig. 1 Proportion of cells in division

b) **The frequency of the phases of mitotic division**

One could notice that all the tests analysed the most important percentage belong to the cells in prophase, followed by those in metaphase, telophase and anaphase.

The frequency of cells in prophase increased after the treatment used comparatively to control, at all the variants, with the exception of the variant 0.01%.

The percentage of the cells in metaphase decreased comparatively to control, at the concentrations applied, the most important decreased being recorded at variant 0.04%.

The cells value recorded an increase at all the variants, in anaphase, this value triplet at the variant 0.03%, comparatively to the control.

The percentage of cells increased at the variant 0.01% and 0.04%, in telophase, comparatively to control, but the high concentration, 0.03% induce significantly decrease (tab.1, fig. 2).

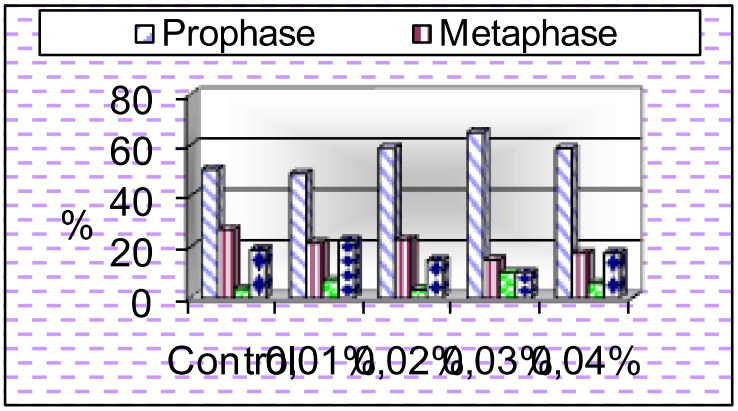

Fig. 2 Proportion of cells in division after the treatment with mutagen agent

c) **The frequency of cells with aberrations**

The treatment with ethidium bromide at *Papaver somniferum L.* didn't significantly change the frequency of cells with aberrant ana-telophases comparatively to control. The percentage of cells with aberration decreased slightly at the variants where the mutagenic treatment was used while this decrease was considerable at the variant 0.02% (36.36%) comparatively to 60.46% to control.

The main types of simple aberration were: ana-telophases with bridges, retarded chromosomes, and with expulsed chromosomes.

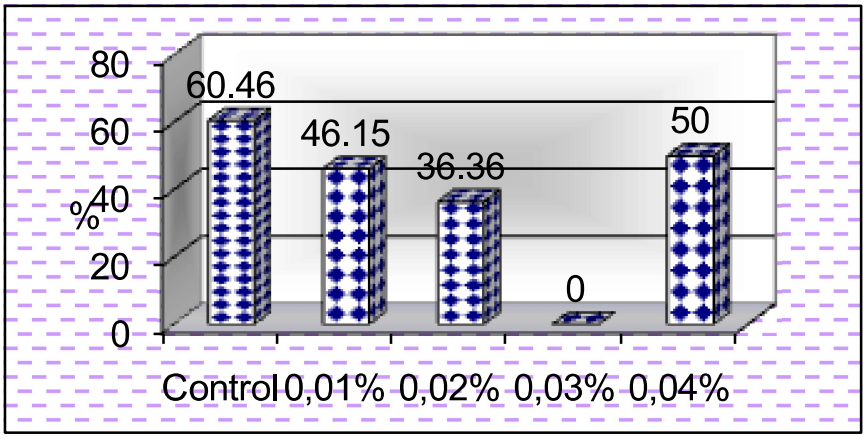

Fig. 3 Proportion of cells in aberrant ana-telophases

CONCLUSIONS

The treatment with ethidium bromide had an inhibitory effect on the process of mitotic division of the cells from the radicular apex, at the species *Papaver somniferum L.*

As concerns the phases of mitotic division, the percentage of the cells in prophase was higher than to those in metaphase, telophase and anaphase, at all variants analysed.

The treatment with ethidium bromide didn't determine the increase of the percentage of cells with aberrations according to literature data, but on the contrary it caused a slight decrease of it, at all the variants analysed.

Table 1

Mitotic index in radicular apex of *Papaver somniferum* after treatment with ehitium bromide

Variant	Total analysed cells	Total cells in interphase		Total cells in mytosis		Cells in mytosis								Mitotic index
						Prophase		Metaphase		Anaphase		Telophase		
		Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	
Control	22592	22402	99.16	190	0.84	96	50.53	51	26.84	7	3.69	36	18.94	0.84
0.01%	18688	18600	99.52	88	0.47	43	48.87	19	21.59	6	6.81	20	22.73	0.47
0.02%	9713	9652	99.37	61	0.61	36	59.02	14	22.95	2	3.28	9	14.75	0.63
0.03%	13360	13340	99.85	20	0.14	13	65.00	3	15	2	10.00	2	10.00	0.15
0.04%	13176	13159	99.87	17	0.12	10	58.82	3	17.64	1	5.89	3	17.65	0.13

Table 2

The frequency of ana-telophases with aberration at *Papaver somniferum* after the treatment with ethidium bromide

Variant	Total ana-telophases	Total aberrant ana-telophases		Types of aberrations in ana-telophases										
				Bridges			Retardatary chromosomes			Expulsed chromosomes			Multi polar A-T	Complex aberration
		Nr.	%	1	2	n	1	2	n	1	2	n		
Control	43	26	60.46	1	4	2	1	2	1	2	-	4	2	5
0.01%	26	12	46.15	8	-	-	-	-	-	-	1	-	-	3
0.02%	11	4	36.36	1	-	1	-	-	-	-	-	-	-	2
0.03%	4	-	-	-	-	-	-	-	-	-	-	-	-	-
0.04%	2	1	50.00	-	-	-	-	-	-	-	-	-	-	1

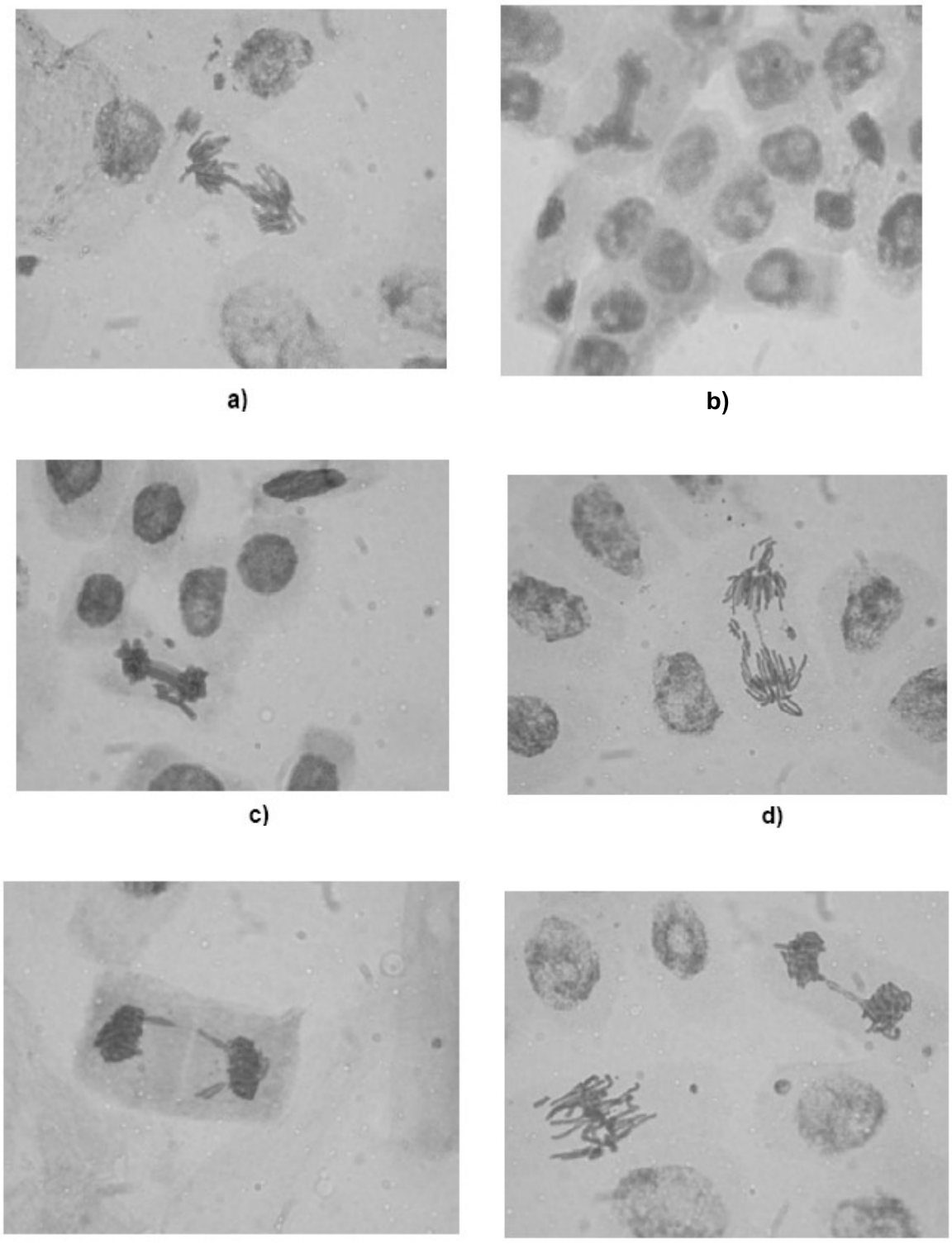

Fig. 1

a) A-T with bridges; b) tripolar A-T and thick bridges; c) A-T with multiples bridges, chromatic material expulsed and fragment; d) A-T with bridges, retarded and expulsed chromosomes; e) Telophases with discontinue bridges and retarded chromosome; f) A-T with double bridge and retarded chromosomes, and interphase with micronuclei.

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