INFLUENCE OF MUTAGEN AGENTS ON DEVELOPMENT OF CAPSULES AND DISTRIBUTION OF ALKALOIDS IN *PAPAVER SOMNIFERUM* L.

INFLUENȚA SUBSTANȚELOR CHIMICE MUTAGENE ASUPRA DEZVOLTĂRII CAPSULELOR ŞI A DISTRIBUȚIEI ALCALOIZILOR LA PAPAVER SOMNIFERUM L.

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Abstract: *In this paper we studied the effect of chemical mutagen agents on capsules development and of alkaloids production on the level of capsules. It was realised the biometry of 50 plants from each repetition, following the diameter and height of capsules, number of stigmatically razes and morphine, thebaine and codeine quantity from the capsules level for selection of the most productive variants.*

Rezumat: *În această lucrare am studiat efectul agenţilor mutageni chimici asupra dezvoltării capsulelor şi a producţiei de alcaloizi de la nivelul capsulelor. Astfel, s-a realizat biometria a 50 de plante din fiecare repetiţie urmărind: diametrul şi înălţimea capsulelor, numărul de raze stigmatice şi cantitatea de morfină, tebaină şi codeină de la nivelul capsulelor, în vederea selectării celor mai productive variante.*

Key words**:** *chemical mutagen agents, diameter, height, capsule, alkaloids.*
Cuvinte cheie: *agenţi chimici mutageni, diametru, înălţime, capsule, alcaloizi.*

INTRODUCTION

Among the medicinal species with a great pharmaceutical value, we can certainly mention the poppy plant (*Papavaver somniferum L.*). The poppy plant contains a series of alkaloids with narcotic properties, which confers its multiple usages.

The investigations regarding the obtaining of some new genotypes, resistant to the unfavourable environment conditions, to diseases and pestilence, more productive as regards the contents of alkaloids were carried out in the 80s, using both mutagen chemical substances and physical factors (Fl. Floria, 1989, Gille Evira, 1989, Băra 1980).

Our experiments refer to the use of some treatments with chemical mutagens with the purpose to increase the genetic variability, the selection and improvement of the contents of alkaloids in the *Papaver somniferum L* species, *Botoşani* population.

MATERIALS AND METHOD

The dry seeds of *Papaver somniferum L.*, *De Botoşani population,* were treated for six hours with three mutagen chemical substances: colchicine, ethidium bromide and acid 2.4-D, in concentrations of 0.01%, 0.02 %, 0.03 % and 0.04%. These seeds were planted in the spring of 2006, on the 25th of March, on the experimental field of The Didactic Station of the Agronomic University Iaşi. The experiment was carried out according to the randomized blocks method, each variant being repeated three times.

In the plants coming from the seeds treated, the results regarding the influence of treatments with chemical mutagen substances were statistically analyzed and interpreted, in the M1 generation, on some morphological and biochemical parameters: the increase in diameter and height of capsules, the number of stigmatic rays and the quantity of alkaloids from the level of capsules through the extraction method of morphine and its isolation through the chromatographic method, after GYÉRESI and RÁCZ (1980). The biometry was achieved at 50

plants for each experiment. The data obtained was processed through the statistical method, determining the arithmetical mean ($\bar{X}$), the standard deviation from the arithmetical mean (sx), the standard deviation (s) and the variability coefficient (s%), the correlation coefficient (r) between some characters.

Table 1

The value of some statistical index at opium poppy capsules after the chemical mutagen treatment, in M1 generation

Variant	The character investigated	Statistical index				Morphine d.s.%	Thebaine d.s.%	Codeine d.s.%
		$\bar{X} \pm s_{\bar{X}}$	s	s %	r			
Control	D	42.43±1.33	9.46	22.31	0.15	0.94	0.47	0.42
	I	50.39±0.99	7.02	13.94				
	No. of stgm. rases	11.34±0.01	0.07	0.64	0.22			
	D/I	1.24±0.42	3.01	22.32				
Colchicine 0.01%	D	31.19±0.21	1.55	4.97	0.11	1.18	0.38	0.43
	I	50.20±0.99	7.01	13.96				
	No. of stgm. rases	12.87±0.01	0.10	0.83	0.27			
	D/I	0.85±0.24	1.72	4.57				
0.02%	D	34.39±0.04	0.31	0.93	0.07	1.09	0.3	0.46
	I	51.67±0.07	0.51	0.98				
	No. of stgm. rases.	12.21±0.09	0.69	5.65	0.43			
	D/I	0.66±0.001	0.008	1.31				
0.03%	D	32.57±0.06	0.46	1.42	0.09	0.89	0.42	0.43
	I	52.90±0.15	1.07	2.02				
	No. of stgm. rases.	12.52±0.08	0.60	4.81	0.32			
	D/I	0.61±0.002	0.014	2.41				
0.04%	D	32.23±0.51	3.65	11.32	0.19	0.86	0.29	0.37
	I	46.82±0.98	6.96	14.87				
	No. of stgm. rases	12.26±0.09	0.67	5.52	0.24			
	D/I	0.70±0.02	0.15	21.33				
Ethidium bromide 0.01%	D	32.73±0.28	2.01	6.15	0.20	0.91	0.35	0.51
	I	50.53±0.99	7.01	13.89				
	No. of stgm. rases	12.50±0.08	0.60	4.81	0.04			
	D/I	0.91±0.27	1.95	7.15				
0.02%	D	42.05±1.39	9.85	23.43	0.27	0.46	0.49	0.44
	I	51.31±0.36	2.57	5.02				
	No. of stgm. rases	12.87±0.03	0.24	1.88	0.54			
	D/I	0.82±0.03	0.22	26.71				
0.03%	D	34.25±0.13	0.95	2.79	0.20	1.14	0.34	0.48
	I	54.58±0.13	0.97	1.79				
	No. of stgm. rases	13.03±0.03	0.22	1.72	0.22			

Variant	The character investigated	Statistical index				Morphine d.s.%	Thebaine d.s.%	Codeine d.s.%
		$\bar{X} \pm s_{\bar{X}}$	s	s %	r			
0.03%	D/I	0.62±0.003	0.02	3.63				
0.04%	D	34.56±0.20	1.41	4.09	0.28	0.99	0.33	0.51
	I	48.10±1.48	10.49	21.80				
	No. of stgm. rases	12.88±0.01	0.08	10.64	0.22			
	D/I	1.01±0.28	1.97	27.27				
2.4-D acid 0.01%	D	31.25±0.22	1.62	5.20	0.13	0.43	0.37	0.53
	I	50.20±0.98	6.98	13.90				
	No. of stgm. rases	12.71±0.06	0.47	3.74	0.01			
	D/I	0.87±0.26	1.85	25.46				
0.02%	D	35.66± 0.07	0.56	1.57	0.10	1.2	0.4	0.45
	I	38.74±0.09	0.68	1.75				
	No. of stgm. rases	12.54±0.07	0.55	4.39	0.16			
	D/I	0.92±0.002	0.02	2.23				
0.03%	D	34.79±0.20	1.45	4.17	0.17	1.16	0.36	0.49
	I	43.69±1.01	7.20	16.49				
	No. of stgm. rases	12.88±0.01	0.12	0.96	0.08			
	D/I	0.81±0.01	0.12	15.84				
0.04%	D	31.10±0.10	0.72	2.34	0.14	1.01	0.35	0.53
	I	53.48±0.20	1.42	2.66				
	No. of stgm. rases	12.52±0.08	0.60	4.80	0.04			
	D/I	0.58±0.003	0.02	3.87				

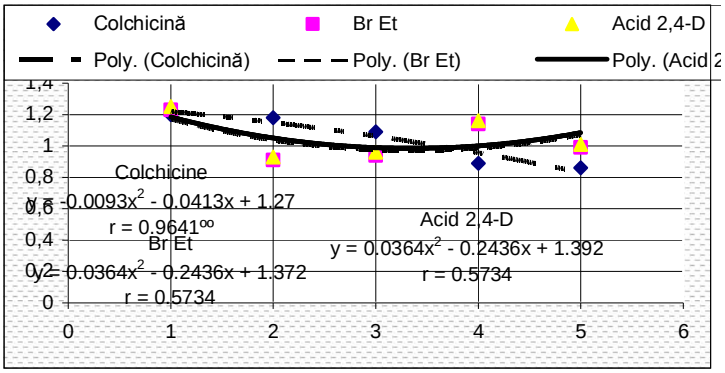

Figure1. Influences of chemical substances on morphine distribution in capsules of *Papaver somniferum*, in M1 generation

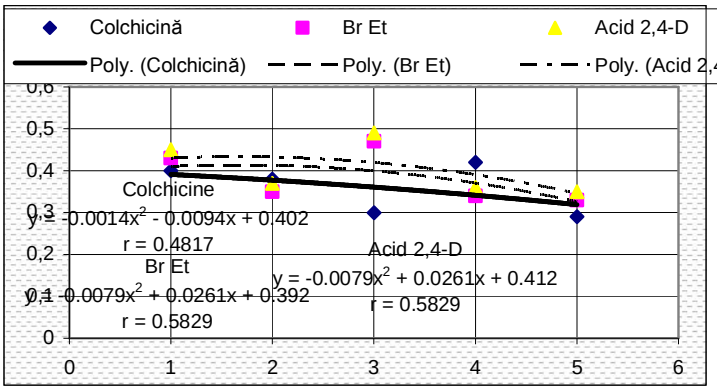

Figure 2. Influences of chemical substances on thebaine distribution in capsules of *Papaver somniferum*, in M1 generation

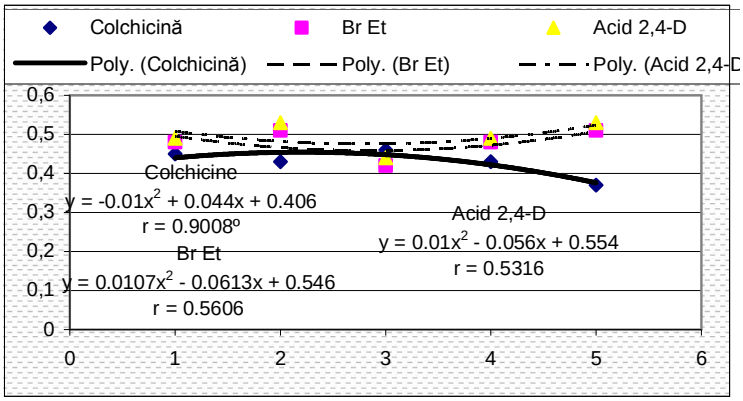

Figure 3. Influences of chemical substances on codeine distribution in capsules of *Papaver somniferum*, in M1 generation

RESULTS AND DISCUSSION

Under the aspect of the capsule form, there are significant differences between the treatment variants. From the point of view of the diameter, the best developed capsules are encountered in the control variant, (42.43), followed by the variant treated with ethidium bromide (42.05). The treatment with mutagen substances, at the concentration of 0.02%, although it inhibited the increase of the diameter of capsules compared to the control, stimulated it compared with the other applied concentrations (table 1).

At the variant treated with colchicine, the smallest average value of the capsule diameter is registered at the variant treated with 0.01% (31.19) colchicine, being followed by the concentration of 0.04% (32.23), while in the variants treated with acid 2.4-D, the smallest average value of the diameter of capsules is encountered in the variant treated with the highest acid concentration 2.4 –D 0.04 % (31.10) being followed by the 0.01%.

Height of the capsule is encountered in the variant treated with ethidium bromide, at the concentration of 0.03% (54.58), and the smallest values, in the variant treated with acid 2.4-D in concentration of 0.02 % (38.74). In the control variant we registered an average value of the height of 50.39. An inhibition of the height growing of capsules was registered at the concentration of 0.01% both in colchicine (50. 20) and in the acid 2.4-D(50. 20), and at the concentration of 0.04 % at colchicine (46.82) and ethidium bromide (48.10).

The treatment with acid inhibited most of all the height growth of capsules in the three types of concentrations applied: 0.01%, 0.02% and 0.03 %, while in the highest concentration, of 0.04% it determined a stimulation of the capsule increase (53, 48) , much over the control average (50,39). This value follows the highest one, of 54, 58, registered in the treatment with ethidium bromide, at the concentration of 0.03%.

The differences between the variants and the control are emphasized, analyzed the behaviour of the capsules' form through the ration between diameter and height.

If in the control variant the ratio between the diameter and height of capsules is of 1.24, therefore very flat capsules, in all the other variants of treatment, we ascertained a much smaller ratio compared to the control, illustrating the presence of longer capsules.

In the treatment with colchicine and ethidium bromide, we ascertain a decrease of the ratio between the diameter and the capsule height beginning with the concentration of 0.01% and until the concentration of 0.04%, where a slight increase is registered. In the variant treated with ethidium bromide 0.04%, this ratio exceeds the values registered in the concentration of 0.01%.

Thus, we can establish that the treatments with colchicine and ethidium bromide induced an increase of the ratio at the concentration of 0.01%, 0.02% and 0.03%, at the same time with an increase of the mutagen concentration, fact that reflects the positive influence on the height. At the variant treated with acid 2.4-D, we established a slight increase of the ratio only at the concentration of 0.02%, and then a decrease of the ratio together with the concentration increase.

It is therefore obvious that, under the influence of the treatment, each type of substance applied behaved in this manner, thus emphasizing the variants treated differently one compared with the other.

Under the aspect of the correlation between the two parameters, we ascertain only positive values, so that these values differ from a type of treatment to the other and differing compared to the control. Thus, in the variant treated with colchicine, the highest coefficient r is encountered in the concentration of 0.04%, followed by 0.01%, and then decreasing in the other concentrations.

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chemical qualities of each, thus differentiating the variants treated differently one from the others.

Under the aspect of the correlation between the two parameters, we determine only positive values, but these values differ from one type of treatment to the other, and also differing from the control. Thus, in the variant treated with colchicine, the highest *r* efficient is encountered in the concentration of 0.04%, followed then by 0.01%, then decreasing in the other concentrations.

In the variant treated with ethidium bromide, the concentration of 0.04% register the highest coefficient, of 0.28, being followed by that of correlation of 0.02%, 0.27. The acid 2, 4-D registers the highest correlation coefficient, at the concentration of 0.03%, 0.17.

The lowest values of the correlation coefficients confirm the idea of a positive correlation between the two dimensions investigated, but in the second one, we establish a slight increase of a dimension and the decrease of the other.

The last morphological parameter taken in the study was the number of stigmatic rays. The highest number of stigmatic rays is registered in the variant treated with ethidium bromide 0.03 %, and the smallest in the control variant. After the treatment with chemical mutagen substances, we ascertained an increase of a number of stigmatic rays compared to the control variant, but without correlating it with the increase of the applied concentrations.

The correlation coefficient between the number of rays and the ratio between the diameter and height of the capsule had the highest values in the variants where the capsule diameter was the biggest.

The production of alkaloids in the poppy plant is strongly influenced by the complex of ecological factors (Floria Fl. 1989), moreover the requirements of the poppy plant as regards the environment from the ontogenetic phase to the other. We even consider there is an interaction of the environment factors with the genetic ones (Băra I., Gille Elvira).

In the variant treated with colchicine, we determine the fact that the contents of morphine decreased together with the increase of the mutagen substance concentration, and the thebaine slightly increased over the value of the control at the concentration of 0.03 %, while the codeine had constant values compared to the control.

The ethidium bromide and the acid 2,4-D determine a decrease of the contents of alkaloids from the capsules for all the treatment values, determining a slightly higher value of the morphine contents in all the variants treated with the concentration of 0.03%, and the codeine registered an increase compared to the control at the concentration of 0.04%.

These values of the alkaloids were statistically verified by calculating the correlation coefficient, after the statistical interpretation determining that the morphine registered a coefficient with a distinctively significant value after the treatment with colchicine 0.01%, and the codeine a significant value after the same type of treatment. (fig. 1,2 and 3).

CONCLUSIONS

The treatment with mutagen substances, at the concentration of 0.02%, although it inhibited the increase of the diameter of capsules compared to the control, stimulated it compared with the other applied concentrations.

In the control variant we registered an average value of the height of 50.39. An inhibition of the height growing of capsules was registered at the concentration of 0.01% both in colchicine (50. 20) and in the acid 2.4-D (50. 20), and at the concentration of 0.04 % at colchicine (46.82) and ethidium bromide (48.10).

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