

**COMPARING SOME BIOLOGICAL PARAMETERS IN *PAPAVER SOMNIFERUM* L. AFTER THE TREATMENT WITH CHEMICAL MUTAGEN AGENTS IN M1 GENERATION**

**COMPARAREA UNOR PARAMETRII BIOLOGICI LA SPECIA *PAPAVER SOMNIFERUM* L. ÎN URMA TRATAMENTULUI CU SUBSTANȚE CHIMICE MUTAGENE ÎN GENERAȚIA M1**

**ANA-MARIA POPA, C. LEONTE**

*University of Agricultural Science and Veterinary Medicine "Ion Ionescu de la Brad", Iași, România*

**Abstract:** *In this paper we present the observation and determination made it in the time of vegetation period of opium poppy and until the moment of plants maturity, in the aim of determining the action of mutagen agents on the opium poppy plants. That's way, it was determined: the percent of emergence plants and survival plants and the frequencies of the chlorophyllian and morphological modifications in M1 generation.*

**Rezumat:** *În această lucrare sunt prezentate observațiile și determinările efectuate în timpul perioadei de vegetație a macului și până la momentul ajungerii la maturitate, pentru a putea determina acțiunea mutagenilor chimici asupra plantelor de mac. Astfel, s-a determinat: procentul de plante răsărite și de plante ajunse la maturitate și frecvența modificărilor clorofiliene și morfologice în generația M1.*

**Key words:** *emergence plants, survival plants, opium poppy, chlorophyll and morphological modifications.*

**Cuvinte cheie:** *plante răsărite, plante mature, mac, modificări clorofiliene și morfologice.*

### **INTRODUCTION**

Knowing the variability of the traits and characteristics in the present species is extremely important when choosing the most appropriate sources of germoplasm and mentioning the directions to follow in the improvement process.

The hereditary traits and characteristics in the poppy plants are encountered in a weaker or stronger dependency, according to their genetic determinism.

In the selection and improvement of plants with economic importance, a first step represents obtaining a very diverse biologic material, and inducing an individual variability of large amplitude. The practice has proven that the tendency towards variability manifested at any species, increases after the intervention with different physical, chemical or biological factors, known under the name of mutagen factors.

### **MATERIALS AND METHOD**

In view of carrying out the treatments with mutagen chemical substances in the *Papaver somniferum* L. seeds, Botoșani variety, we chose four variants (0.01%, 0.02%, 0.03%, and 0.04%) for each applied substance. Each variant has been repeated three times.

The biologic material chosen in this manner was prepared in advance, the seeds treated with chemical mutagen substances being then dried, in order to seed in the optimal period and under appropriate conditions for the germination of treated seeds.

In order to test the resistance of biological forms taken in the study and also, to provoke a variability as high as possible within them, the seeds were treated with three mutagen chemical substances : colchicine, ethidium bromide and acid 2.4-D, each substance

having four concentrations : 0.01%, 0.02%, 0.03%, 0.04%. These solutions acted on the seeds of *Papaver somniferum* for six hours.

After the treatment with mutagen chemical substances, the seeds were directly seeded in the experimental field of the Plant Improvement subject from the Didactic Station Adamachi Iași. The seeding was achieved on the 25<sup>th</sup> of March 2006, manually, at distances of 10 cm between the seeds on the row and 30 cm between the rows.

The plants resulted constituted the first mutagen generation (M1), in this field carrying out the first observations and determinations. We applied no chemical treatment on the soil or plants, the experiment being kept clean of weeds by clearing them constantly, by manual hoeing, thinning, anytime it was necessary.

The plants from the M1 generation were subject to observations and determinations during the vegetation period and the moment they reached the maturity, for determining the action of chemical mutagens on the poppy plants, by using some tests recommended by the specialized literature thus, we determined: the percentage of plants emerged and the percentage of plants reaching maturity and the frequency of their morphological modifications.

The degree of emergence and survival of the plants from the four studied variants was determined by counting the emerged plants, which respectively reached the maturity and their correlation to the number of seeds treated with seeded chemical substances, for each variant in part, and also by reporting the number of emerged plants (respectively aged) from the seeds treated at the number of plants emerged of the untreated control.

The appearance frequency of the morphological modifications in the M1 generation was determined by reporting them to the number of emerged plants, for each variant in part.

The data obtained after the observations and determinations carried out were processed statistically according to the consecrated models, mentioned in the specialty literature (Săulescu et al. 1967, Ardelean, 1994, Leonte 1997).

In the M1 and M2 generations, the sensitivity to mutagen agents of De Botoșani variety was determined by repeating the experiment three times, by calculating the degree of emergence and survival in percentages and graphically representing the equations of the square regression curves and the frequencies of manifestation of the chlorophyll and morphological modifications (M1). The significance of the difference between the variants treated and the control were determined through the method of limit differences (DL 5%, 1% and 0.1 %).

## **RESULTS AND DISCUSSION**

The emergence degree of poppy plants in the field, in comparison with the untreated control, reflects rather well the sensitivity of the biological material in the treatment with mutagen chemical substances.

In table 1 we present the effects of the treatments with chemical mutagen substances on the emergence degree of *Papaver somniferum L* plants, for all the administered substances and the variants of experimentation.

In comparison with the witness, represented by the untreated seeds of *Papaver somniferum* species, in which the plants emerged in an average percentage of 95.43%, in the untreated variants, the plants emerged in stages and in a non-uniform manner, approximately in inverse ratio to the size of administered concentrations.

In the variant treated with colchicine, the concentration of 0.01 % did not bring any significant reduction of the emergence degree of plants, being of 89.7%, respectively 94.72 % compared to the untreated control. In exchange, the concentration of 0.02 % determines a significantly distinctive reduction of the percentage of emerged plants ( 84.2 of the planted seeds, respectively 88.9 % compared to the control variant), and when applying the concentrations of 0.03 % and 0.04 %, the emergence degree of plants is significantly reduced,

to 78.6% and respectively 52.4 % ( 83.0% and respectively 55.33 % compared to the untreated control).

In the variant treated with ethidium bromide the concentration of 0.01 % determined a distinctly significant reduction of the percentage of emerged plants, the emergence degree being reduced then while the administered concentrations increase, the differences from one concentration to the other being ensured statistically.

The variant treated with acid 2,4- D, where an average percentage of emergence was registered, in the control variant of 95.4%, the action of the treatment with the concentrations of 0.01 % and 0.02%, the differences compared to the control variant, untreated , being significant and respectively distinctly significant.

Table 1

Influences of mutagen chemical substances treatments upon the emergence degree of plants in the field, in M1 generation (% front of number of sow seed)

Substances	Colchicine	Ethidium bromide	2,4-D acid
Control	94.7	96.2	95.4
0.01 %	89.7	95.1 <sup>oo</sup>	94.5 <sup>o</sup>
0.02 %	84.2 <sup>oo</sup>	94.7 <sup>ooo</sup>	94.0 <sup>oo</sup>
0.03 %	78.6 <sup>ooo</sup>	66.2 <sup>ooo</sup>	55.2 <sup>ooo</sup>
0.04 %	52.4 <sup>ooo</sup>	57.6 <sup>ooo</sup>	49.0 <sup>ooo</sup>

DL 5%            6.3                    0.6                    0.8

DL 1%            9.2                    0.9                    1.2

DL 0.1%        13.8                  1.3                    1.8

Thus, all the three types of treatments are similar to DL 50, in the concentration of 0.04 % and respectively 0.03 %, the variant treated with ethidium bromide being more resistant, having 57.6 % emerged plants and the variant treated with acid 2,4 D, in the concentration of 0.04 % are situated under the level DL 50 (fig. 1).

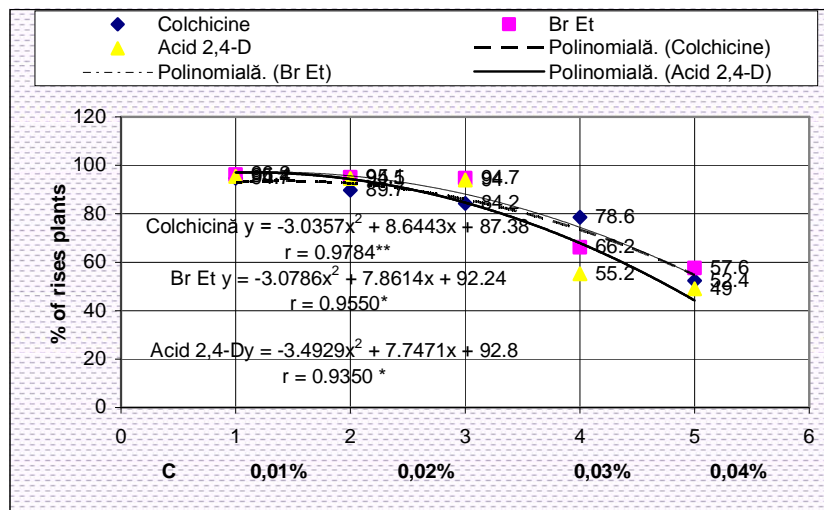


Figure 1 Influences of mutagen chemical substances treatments upon the emergence degree of plants in the field, (M1 generation)

The correlations between the degree of plant emergence in the field and the value of concentrations were positive, the correlation coefficient having significant values for the treatments with ethidium bromide and acid 2,4- D and distinctively significant with colchicine.

In table 2, we presented the data regarding the frequency of chlorophyll modifications, ensured statistically, the percentage determinations being made in the number of emerged plants, for each type of treatment applied. We mention that the individuals which presented serious chlorophyll deficiencies presented little progress regarding the vegetation, after which they no longer managed to survive.

*Table 2*

Frequencies of chlorophyll modification after the mutagen chemical substances, in M1 generation (% front of number of emergence plants)

Substances	Colchicine	Ethidium bromide	2,4-D acid
Control	0	0	0
0.01 %	2.58	3.9 <sup>xx</sup>	2.5 <sup>x</sup>
0.02 %	4.23 <sup>x</sup>	5.2 <sup>xx</sup>	4.6 <sup>xx</sup>
0.03 %	5.56 <sup>xx</sup>	6.0 <sup>xx</sup>	8.2 <sup>xxx</sup>
0.04 %	6.8 <sup>xx</sup>	9.5 <sup>xxx</sup>	9.3 <sup>xxx</sup>
<i>DL 5%</i>	3.2	2.0	2.0
<i>DL 1%</i>	5.3	3.3	3.3
<i>DL 0.1%</i>	9.9	6.2	6.2

In the variant treated with chlorophyll, the maximum frequency of the chlorophyll deficiencies, checked statistically, was registered at the concentration of 0.04 % (6.8%), and in the variant treated with the concentration of 0.03 %, it decreased at 5.56 %, being also distinctly significant. The treatments with ethidium bromide and acid 2,4-D determined a higher sensitivity of the poppy plants. At the concentrations of 0.04\$, the ethidium bromide substance determined a higher sensitivity of plants to these treatments, the frequency of chlorophyll modifications of 9.5%, this value being the highest of all the registered ones, very significant value from the statistical point of view.

From the data presented, the variant treated with acid 2,4-D is emphasized, where we obtain a frequency of modifications of 9.3% and 8.2%, the values being very significant and with a highest sensitivity of plants to this treatment, the frequency of chlorophyll modifications in the four types of treatment, in general, being maximum at the concentration of 0.04%, followed closely by the concentration of 0.03%.

These results obtained in our experiments confirm the data obtained by Băra and Floria, 1980, which consisted in an experiment with the poppy plants treated with mutagen chemical substances that, in M1 generation, the highest frequencies of plants with chlorophyll deficiencies are registered in the moderate treatment concentrations.

The most frequent morphological modifications were: the modified, deformed leaves, rarely fasciations of the stem and dwarfing of plants. In comparison with the frequency of chlorophyll modifications, the morphological ones had an obviously higher frequency, as we can notice from table 3.

In addition, this time the treatments with ethidium bromide and acid 2,4-D have proven to have a stronger effect on poppy plants, which present a much higher sensitivity compared to those treated with colchicine. Thus, in the treatment with ethidium bromide, the

concentrations of 0.03% and 0.04 % determined a very significant percentage of morphological anomalies (19.36 % and respectively 7.0%).

According to the statistical data, the variant with 0.03 % of the acid 2,4-D determined the highest frequency of morphological modifications, the value registered being very significant. At the concentration of 0.02% and 0.04% we registered distinctly significant values of the morphological modifications frequency.

Table 3

Frequencies of morphological modification after the mutagen chemical treatment, in M1 generation (% front of number of emergence plants)

Substances	Colchicine	Ethidium bromide	2,4-D acid
Control	0.2	0.2	0.2
0.01 %	2.8 <sup>x</sup>	2.0 <sup>x</sup>	2.1 <sup>x</sup>
0.02 %	2.2	2.9 <sup>xx</sup>	2.8 <sup>xx</sup>
0.03 %	17.7 <sup>xxx</sup>	19.3 <sup>xxx</sup>	22.5 <sup>xxx</sup>
0.04 %	8.6 <sup>xxx</sup>	7.0 <sup>xxx</sup>	3.1 <sup>xx</sup>
DL 5%	2.2	1.3	1.4
DL 1%	3.7	2.2	2.2
DL 0.1%	6.9	4.1	4.2

In the other two variants, treated with ethidium bromide and colchicines, the highest frequency of anomalies is noticed at the concentration of 0.03 % (19.4% and respectively 17.7%), both values being very significant.

In order to have a more relevant image regarding the sensitivity of cultivations, the researchers recommend assuming the frequencies of all the types of modifications (Nicolae, 1978, Țirdea , 1984).

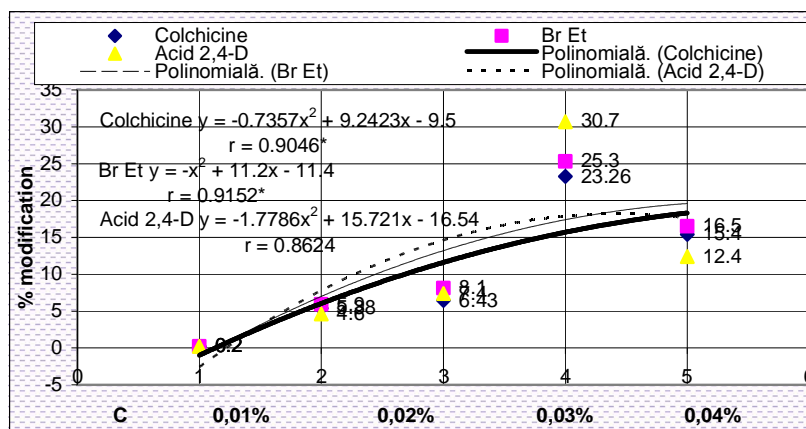


Figure 2 Influences of chemical mutagen treatment upon the frequencies of chlorophyll and morphological modification (M1 generation)

From the graphical representation of the regression equations (figure 2), we notice that the total frequency of modifications differed from one substance to the other, the highest values being registered in the variant treated with acid 2,4 -D , at the concentration of 0.03

(30.7%). The maximum frequency in all the types of treatment was determined by the concentration of 0.03 %.

The correlations between the frequency of modifications and the concentrations of chemical substances administered have had positive values, un-ensured statistically for the variant treated with acid 2,4-D and statistically ensured for the variants treated with ethidium bromide and colchicine, having non-significant values.

In the M1 generations, the plants resulted from the untreated seeds, respectively from the control variants of each type of treatment, have reached maturity in relatively similar proportions, compared to the number of emerged plants. The smallest degree of survival was registered in the plants treated with colchicine, respectively 88.4 %, while in the variant treated with ethidium bromide 91.9 % of emerged plants survived. We consider that the differences compared to the 95.4 % emerged plants (in average, in all the control variants of the four types of treatment), were due to unfavourable conditions of environment from the vegetation period.

In table 4, we present the results regarding the survival degree of plants at the end of the vegetation period, in all the four treatments, for all the experimental variants, in percentages compared to the number of treated individuals (respectively the number of planted seeds), which was the same for each variant, in comparison with the untreated control.

Table 4

Influences of chemical mutagen treatment upon the degree of survival plants in the field, in M1 generation

Substances	Colchicine	Ethidium bromid	2,4-D acid
Control	88.4	91.9	90.5
0.01 %	87.0	90.0	88.8
0.02 %	82.3 <sup>000</sup>	88.2 <sup>00</sup>	87.5 <sup>0</sup>
0.03 %	74.3 <sup>000</sup>	62.3 <sup>000</sup>	52.2 <sup>000</sup>
0.04 %	49.5 <sup>000</sup>	51.4 <sup>000</sup>	43.2 <sup>000</sup>
<i>DL 5%</i>	1.7	2.1	2.4
<i>DL 1%</i>	2.4	3.0	3.5
<i>DL 0.1%</i>	3.6	4.6	5.3

In the variant treated with colchicine, if the plants survived in a percentage of 88.4%, in the untreated control variant, in the concentration of 0.02 % the survival degree of plants was reduced very significantly, at 82.3%, and at the concentration of 0.04 % only 49.5 % of the plants reached a maturity, the difference of 38.9 % compared to the untreated control being very significant. Through extrapolation, we obtained the level DL 50, and the variant treated with colchicine is situated very low under the concentration of 0.04% (fig. 3).

The plants treated with acid 2,4-D have proven to be more sensitive at the action of this chemical substance. If the plants of the control variant that we compared with the other variants of chemical treatment of the acid substance 2,4-D reached the maturity in proportion of 90.5%, the concentration of 0.02% determined a significant reduction of the percentage of plants which survived, respectively 87.5%, and the concentrations of 0.03 and 0.04% determined a very significant reduction of the percentage of plant survival, of 52.2 % and respectively 43.2%, this last value being much under the level DL 50.

The variant treated with ethidium bromide registered, at the concentration of 0.02 % a distinctly significant reduction of the percentage of surviving plants, and at the concentration of 0.04 % it did not exceed the threshold of DL 50, the plants surviving in a percentage of

51.4%, compared to the untreated control which registered a difference 40.5%, very significant.

At the concentration of 0.01%, for all the three substances used, the percentage of survived plants significantly decreases.

The regression curves between the degree of plants' survival and the concentrations administered (figure 3), in the first post-treatment generation, emphasizes a rather similar reaction of the three chemical substances used when applying the increasing concentrations, the values of correlation coefficients being positive and distinctly significant.

In addition, we notice the fact that the treatment with the concentration of 0.01 % did not have a stimulatory effect in any of the variants regarding the plants' survival, the effect being still a reduction of the percentage of survived plants, but to a smaller extent, non-ensured statistically.

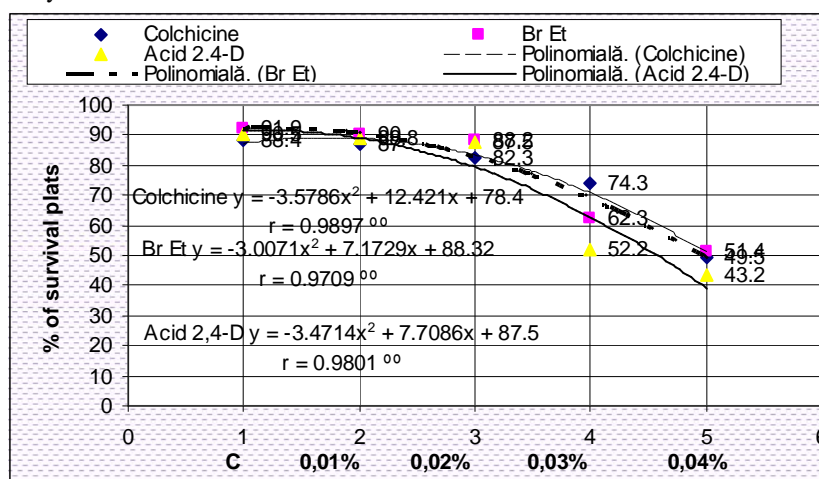


Figure 3 Influences of chemical mutagen treatment upon the survival degree of plants in the field (M1 generation)

## CONCLUSIONS

In comparison with the witness, represented by the untreated seeds of *Papaver somniferum* species, in which the plants emerged in an average percentage of 95.43%, in the untreated variants, the plants emerged in stages and in a non-uniform manner, approximately in inverse ratio to the size of administered concentrations.

All the three types of treatments are similar to DL 50, in the concentration of 0.04 % and respectively 0.03 %, the variant treated with ethidium bromide being more resistant, having 57.6 % emerged plants and the variant treated with acid 2,4 D, in the concentration of 0.04 % are situated under the level DL 50.

The correlations between the degree of plant emergence in the field and the value of concentrations were positive, the correlation coefficient having significant values for the treatments with ethidium bromide and acid 2,4- D and distinctively significant with colchicine.

From the data presented, the variant treated with acid 2,4-D is emphasized, where we obtain a frequency of modifications of 9.3% and 8.2%, the values being very significant and with a highest sensitivity of plants to this treatment, the frequency of chlorophyll modifications in the four types of treatment, in general, being maximum at the concentration of 0.04%, followed closely by the concentration of 0.03%.

The most frequent morphological modifications were: the modified, deformed leaves, rarely fasciations of the stem and dwarfing of plants. In comparison with the frequency of chlorophyll modifications, the morphological ones had an obviously higher frequency

The total frequency of modifications differed from one substance to the other, the highest values being registered in the variant treated with acid 2,4 -D , at the concentration of 0.03 (30.7%). The maximum frequency in all the types of treatment was determined by the concentration of 0.03 %.

The regression curves between the degree of plants' survival and the concentrations administered, in the first post-treatment generation, emphasizes a rather similar reaction of the three chemical substances used when applying the increasing concentrations, the values of correlation coefficients being positive and distinctly significant.

### LITERATURE

1. FLORIA FL. BĂRA I.– Efecte ale tratamentului cu EMS la patru soiuri de mac, în M1, Lucrările Stațiunii „Stejarul”, Ecologie terestră și genetică, Pângărați, 1980, 229-137.
2. JIȚĂREANU GERARD – Tehnică experimentală, curs, USAMV, Iași, 1994, 142-153.
3. LEONTE CONSTANTIN – Ameliorarea plantelor, Ed. Ion Ionescu de la Brad, Iași, 2003.
4. LEONTE CONSTANTIN – Ameliorarea plantelor horticole și tehnică experimentală, lucrări practice, Ed. Univ. Agronomice Iași, 1997.
5. MIRICIOIU ECATERINA, BĂRA I., FLORIA FL. – Comportarea unor variante de *Papaver somniferum* L. la tratamentul cu EMS și DES, în M2, Lucrările Stațiunii Stejarul, Ecologie terestră și genetică, 1980, 255-261.
6. MORARIU ALIONA – Teză doctorat – Cercetări privind efectele citogenetice și biochimice induse de tratamente cu agenți alchilanți la specii ale familiei Papaveraceae.
7. NICOLAE, I. – Mutagenză experimentală, Ed. Ceres, București, 1978, 37-40, 42-43.
8. ONISIE T., JIȚĂREANU G. – Tehnică experimentală, lucrări practice, USAMV, Iași, 1992, 29-51.
9. SNEDECOR, G.W. – Metode statistice aplicate în cercetările de agricultură și biologie, Ed. Didactică și Pedagogică, București, 1968, 48-91.