ABSTRACT

of doctoral thesis

***„Researches about the mutagen agents' effect on morpho-phisiological and biochemical characters at Papaver somniferum* L."** elaborated by **Popa Ana – Maria**, under the coordination of PhD, Prof. **Constantin Leonte**, at „Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iaşi, Horticultural Faculty.

The paper is structured on 5 chapters, covers 81 tables and 113 figures.

In the first part of the thesis it was made a summary synthesis of the researches made at *Papaver somniferum* species, in the country and outside of it, regarding to morpho-phisiological biochemical and cytogenetically characterization and also a characterization of natural frame, during the experiments.

In the second part are presented the own research results made during the doctoral work, being included the chapter that make reference followed goals, the biological material and research methods used to fulfill the aim of these thesis.

For the elaboration of the paper and the interpretation of the results it were consulted 196 bibliographical titles, some of which constitute pioneering work, others with monographic character, necessary for knowing the breeding, experimental techniques, genetically, morphology, physiology and biochemical aspects of the studied species.

The experiments display in the field condition of Ezăreni Didactical Stations from Iaşi have the principal goal to increase the variability of opium poppy germoplasm source for opium (*Papaver somniferum* L., De Botoşani local population) by inducing the mutations using the chemical substances, watching the behavior of some mutant in the aim of identifying the most productive biological forms, the increased alkaloids content, and eventually, the precocity, all of these in the aim of obtain some families which could be proposed to homologation and recommend in production.

The selection in the experimental field was made using the method of half seeds (Munteanu, 2000), typically used for alogame plants such it is the *Papaver somniferum*.

Proceeding with the general scheme of selection by half seeds method the opium poppy elites was individually choused from the first tow generations, in the first season (CA) and respectively in the first selection field (CS_1).

For surprise the sensibility of species at the mutagen action we made the divers attempts in the experimental field and labs. The physiological, biochemical and cytogenetically methods were realized in divers lab's from the „Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, Iaşi and outside of it.

The dates obtained after the observation and determination made had had the statistical analysis proceeding with devoted methods mentioned in the specialty literature. In M_1 and M_2 generations the De Botoşani variety sensibility at mutagen agents were determinate in three repetitions calculating the rises grade and plants survival percentage and graphical representation of regression square bend and also the frequency of chlorophyll and morphological modifications (M_1), and respectively the frequency of chlorophyll mutations (M_2). The signification difference between the control and treated variants were determinate by limits difference method (DL 5%, 1% şi 0.1%). The quantitative characters were statistically analyzed with variance analyses and variability coefficient. In M_3 generation were determinate the medium values of quantitative characters ($\bar{x}$) and variability coefficient (s%), and the differences significations between control and treated variances were made by limits difference method (DL).

For testing the biological forms resistance and also for bring a biggest variability of those, the seed were being treated with three chemical mutagen substances, each substance having four dozes: 0.01%, 0.02%, 0.03%, 0.04%. These solutions have had suing on the seeds of *Papaver somniferum* L. six hours.

As far it is known, these mutagen agents have the capacity to increase the chromosomical restructuration frequency, to reduce the seed germination capacity, the growing plants and the survival grade.

In our researches we propose the selection and to make evident some of the good mutant families from quantitative and qualitative point of view.

It was investigated some morphological particularities because we consider that the phenotyping the morphological traits of capsules and seeds, genic determinate, after the chemical mutagen substances treatment, could represent a supplementary criterion for selection and for obtain some mutant families which could be more productive.

In our experiments, we noticed the diminution of the number of germinated seeds once with the increase of the amount of mutagen agent used for all the treatment variants. As compared to the blank test, represented by untreated seeds of the species *Papaver somniferum*, for which plants sprang in an average percentage of 95.43%, for the treated variants the plants sprang in stages and in a non-uniform manner, approximately in values inversely proportional to the size of the amounts administered. All the three types of treatments are close to DL 50, for the dose of 0.04% and 0.03%, respectively, the variant treated with ethidium bromide being more resistant and having 57.6% sprung plants, and the variant treated with 2.4-D acid, with a dose of 0.04% situates below the DL 50 level.

In M_1 generation, the plants resulted from untreated seeds and from the blank test variants of each treatment type reached maturity in proportions relatively similar as compared to the number of sprung plants. The lowest level of survival of the poppy plants was registered following the colquicine treatment, namely 88.4%, whereas for the variant treated with ethidium bromide 91.9% of the percentage of sprung plants survived. The regression curves between the degree of survival of plants and the administered doses highlight a quite similar reaction of the three chemical substances used to apply the increasing doses, the values of the correlation coefficients being positive and distinctly significant.

Once increasing the action dose of mutagen, for the treatments with ethidium bromide and colquicine we noticed a directly proportional increase of plant height, except the biggest dose, of 0.04%, for which we registered a decrease of the values of plant height.

The analyzed variants had big and very big values of the variability coefficients for the capsule weight, what suggests that the chemical treatment represents an important criterion for the selection of the possible mutant forms.

As for the seed weight, we could notice an increase of it as compared to the blank test variant treated with ethidium bromide, the increase occurring once increasing the mutagen dose. As for the correlation between the two parameters, the weight of capsules per plant and the weight of seeds per capsule, we notice positive values meaning that the capsule weight and seed weight are in an interconditioned variability.

The treatments with colquicine and ethidium bromide induced an increase of the ratio for the doses of 0.01%, 0.02% and 0.03%, concomitantly with an increase of the mutagen dose, a fact reflecting the positive influence over height. For the variant treated with 2.4-D acid we noticed a slight increase of the ratio only for the dose of 0.02%, and then a decrease of ratio once increasing the dose.

For all the three types of treatment applied to plants we registered values of the correlation coefficients modified both by applying the different doses of treatment and by the mutagen substance used for the analyzed characters.

In M_1 generation, in case of the treatment with 2.4-D acid, we noticed very few correlations of negative order as compared to the other two treatments. Thus, in M_1 generation, we may say that 2.4-D acid determined generally a positive relation between the hereditary characters causing a stimulation of growth and development of *Papaver somniferum L* plants.

In M_2 generation, the percentage of sprung plants reduces once with the increase of mutagen dose but, as compared to M_1 generation, this reduction is not as drastic.

The values of correlation coefficients, distinctly significant, for all the treatment types applied, indicate the existence of some negative correlations between the percentage of sprung plants in M_2 generation and the mutagen doses.

The percentage of survived plants, as compared to M_1 generation, for the variants treated with the three mutagens was high but dependent again on the dose of mutagen applied. In terms of the degree of plant survival, in M_2, the plants treated with 2.4-D acid were the less sensitive and the ones treated with ethidium bromide were the most sensitive.

For the treatments with colquicine and ethidium bromide, the plant height increases progressively once increasing the mutagen dose, whereas for the treatment with 2.4-D acid the plant height decreases inversely proportional to the mutagen dose. The biggest average height of plants was registered for the variants with ethidium bromide.

Generally, the highest weight of capsules was registered for the dose of 0.03%, and the intensity of this effect decreased for the highest dose of 0.04%.

The seed weight per capsule registered increases for the treatment with colquicine and ethidium bromide, whereas the treatment with 2.4-D acid caused a diminution of the seed weight per capsule.

The correlation coefficient between the two parameters analysed, capsule weight per plant and seed weight per capsule was higher than for the blank test in most studied cases, showing a parallelism of their variability, the direct proportionality and their interdependence. Variability of a parameter in one sense draws the variability of another to the same sense.

The correlation between the number of rays and the ratio diameter-height generally has positive values in the sense that the number of ray is higher, the bigger the diameter of capsules is.

The character most affected by mutations was the type if growth, with a frequency of mutants with voluble increase of 0.85%, followed by the modified color of capsules (0.28% of the entire M_2 generation).

The mutant plants for the qualitative characters did not stand out by superior values of the quantitative characters of interest in determining productivity. They were withheld and studied to identify genetic determinism – dominant or recessive – of the affected characters.

Taking into account the mutant elites both for quantitative and qualitative characters, for all the three mutagen substances applied, it resulted an average value of selection pressure of 7.79%.

The biometric determinations effectuated on the 19 mutant families for qualitative characters, in M_3 generation, allowed their appreciation as valuable only from the theoretical

viewpoint; none of these families managed to register satisfying values of he components elements of qualitative characters.

From the 106 M_3 families, considered mutant for quantitative characters, we noticed and withheld only 7 families to be tested in the test field, namely those that cumulated inferior values of variability coefficients as against the untreated blank test, manifesting as stable in terms of useful micro mutations induced by the treatment with mutagen chemical agents. The families withheld are: B-c-3-7.4-07; B-c-4-9.2-07; B-b-1-9.7-07; B-b-2-17.1-07; B-b-4-2.6-07; B-a-2-5.1-07; B-a-3-8.6-07.

The analysis of average weight of seeds per capsule of the mutant families obtained following the treatment with mutagen chemical substances, in the selection field, confirmed for the 7 families tested, a real superiority as against the untreated blank test, superiority expressed by differences of seed weight per capsule from distinctly significant to very significant.

By the morphological investigations we made the physiological, biochemical and cytogenetically methods which make contribution to selection of the best mutant families in the aim of making a new harvest.

The obtained results can sustain the idea of using the chemical substances in reduced dozes, followed by a rigorous selection in mutant generation, to obtaining some new genotypes, superior of untreated control.

From the analysis of the results obtained following the researches effectuated in the interval 2005-2008, that mainly aimed at creating new valuable genotypes by induced mutagenesis on poppy, we noticed that the application of different doses of mutagen substances triggered the phenotypic manifestation of some important modifications of the characters studied for the plants of *Papaver somniferum* L., De Botoşani species.

Thus, we may assert that for this species, the treatment with mutagen chemical substances is an efficient method to increase genetic variability and to obtain some new biological forms.