UDC: 575.224.46.044 DOI:10.36710/ioc-2021-30-01

THE EFFECT OF CHEMICAL MUTAGENS ON THE CHARACTERISTICS OF PLANTS *NIGELLA DAMASCENA* L. VARIETY BEREGINYA IN M_1 GENERATION

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We studied the effect of chemical mutagens EMS, NMU and a new mutagen DG-2 on the traits of *Nigella damascena* L. variety Bereginya in the generation M_1. It was found that the treatment of love-in-a-mist seeds with these substances changed such characteristics as plant survival, plant height, and the number of seeds per plant. The greatest influence on these indicators was exerted by nitrosomethylurea. In our study, all chemical mutagens led to a decrease in plant survival and mainly caused plant oppression. In all treatments, a decrease in the total plant height was revealed in comparison with the control. In some cases the number of seeds per plant has decreased. The tested mutagens caused the appearance of morphoses in plants with a frequency of 3.87% to 0.48%, characterized by a change in color and shape of the leaves and the plant dwarfism. The appearance of plants with altered characteristics in the M1 generation assumes hereditary mutations of a different nature in the subsequent generations.

Keywords: *Nigella damascena,* love-in-a-mist, mutagenesis, M_1 generation, EMS, NMU, DG-2, morphose, survival rate, morphometric characteristic.

Introduction. Recently, there has been an increase in interest in new crops with economically valuable traits. These include *Nigella damascena* L., also known as nigella or love-in-a-mist, from the genus *Nigella*. The genus *Nigella* (*Nigella* L.) belongs to the Buttercup family (*Ranunculaceae* Juss.) It comprises around 20 species common in the countries of the East and Southern Europe, the Caucasus, and Central Asia (Hosseinzadeh, 2014). The species *Nigella damascena* L. has a wide range of useful properties. It is gaining more and more importance as a medicinal, spicy-aromatic, essential oil plant in the field of medicine, ornamental gardening, in the food industry (Sieniawska, 2018). To increase the growing area under nigella it is necessary to develop new highly prolific varieties with valuable traits that meet the requirements of commodity producers and raw material processors.

Breeding methods can improve the crop, for example, increase its yield potential and adaptability. According to literature data average seed yield of *Nigella damascen*a L. ranged from 172.1 g/m^2 (Isakova et al, 2015), to 277.2 g/m^2 and 310.0 g/m^2 in two varieties of Belarusian selection Radast and Sunichny Vodar respectively (Prokhorov et al, 2020). In production crops yield of closely related species *N. sativa* ranges from 0,9 to 1,0 t/ha thus providing ample opportunities for breeding (Kizil, 2005).

One of the methods capable to expand the range of hereditary variability in any plant species is induced mutagenesis, chemical mutagenesis in particular. The use of

experimental mutagenesis allows to effectively change the genotype of a cultivated plant, to improve specific traits, and obtain new properties and traits that are not found in nature (Lyakh et al, 2009). Experimental chemical mutagenesis, due to its comparative availability and low cost, is still a relevant and effective breeding tool today.

The aim of this work was to study the survival rate, the frequency of morphoses, and the variability of some morphometric characteristics of plants of the nigella variety Bereginya in the M_1 generation under the influence of various chemical mutagens.

Material and Methods. The air-dry seeds of the Bereginya variety were treated with chemical mutagens - ethyl methanesulfonate (EMS), nitrosomethylurea (NMU) and a new chemical mutagen DG-2 (a complex of 3-N, N-dimethylaminosulfolane with dimethyl sulfate) (Tigova, Soroka, 2016). In each variant 350 seeds were treated in one replication. Seeds in cotton bags were soaked in 0.01 and 0.05% aqueous solutions of EMS and NMU mutagens, and 0.5 and 0.05% aqueous solutions of DG-2. As a control seeds of the corresponding variety were used, which were soaked in distilled water. The treatment exposure was 6 and 16 hours. After the treatment, seeds of each variant were washed for an hour under running water, and on the same day they were sown in 2.5 m long rows with 20 cm spacing. Before flowering, nigella plants were isolated with individual micro-perforated polypropylene pollination bags. Observation of plants in experimental and control treatmens was carried out in the experimental plots of the Institute of Oilseed Crops of the National Academy of Agrarian Sciences of Ukraine.

To analyze the effect of the mutagen on plants in the M_1 generation, we studied such characteristics as plant survival rate, the presence of morphoses, as well as a number of morphological traits (plant height, number of lateral shoots on the main stem, number of capsules per plant, and number of seeds per plant). During the growing season phenological observations were carried out, counted plants with altered morphological characteristics. Morphoses were isolated by visual inspection of plants during the main phases of the vegetation period. Harvesting from the experimental plots was carried out manually. The number of surviving plants was counted 6 weeks after sowing.

The observation results were calculated using standard mathematical and statistical methods (Natan et al, 2004). The main statistical characteristics of the quantitative change in the studied parameters were the arithmetic mean ($\bar{x}$), variance (s^2), standard deviation (s), and the standard error of the mean (s_x). The significance of deviations of the experimental data from the control was evaluated by the Student's t-test.

Results and their Discussion

Evaluation of the response of plants in the M_1 generation to the action of mutagens is necessary to determine the degree of toxicity of the mutagen and the sensitivity of plants, optimal and critical doses, in order to rationally use the minimum doses of the mutagen for maximum efficiency. When studying the effect of chemical mutagens on plants of *Nigella damascena* L. in the first generation, the sensitivity of plants to the influence of ethyl methanesulfonate, nitrosomethylurea at the concentrations of 0.01 and 0.05%, and DG-2 at the concentrations of 0.05 and 0.5% was assessed. It was found that the mutagenic factor influenced such an important characteristic as “plant survival rate” in the M_1 generation (Table 1). Studies have shown that plant survival significantly depended on the type of mutagen.

Nitrosomethylurea had the greatest effect on plant survival. After treatment with this mutagen at the maximum concentration of 0.05% and the maximum exposure

Table 1

Influence of chemical mutagens on the survival rate of *Nigella damascena* L. plants variety Bereginya in the M_1 generation

Mutagen	Mutagen concentration , %	Exposition, hour	Number of survived plants, %
EMS	0.01	6	47.1±2.67*
EMS	0.01	16	53.4±2.67*
EMS	0.05	6	54.3±2.66*
EMS	0.05	16	42.9±2.65*
NMU	0.01	6	44.3±2.66*
NMU	0.01	16	29.7±2.44*
NMU	0.05	6	16.6±1.99*
NMU	0.05	16	2.9±0.90*
DG-2	0.05	6	58.3±2.63*
DG-2	0.05	16	53.1±2.67*
DG-2	0.5	6	61.1±2.60*
DG-2	0.5	16	59.4±2.63*
Distilled water		16	66.0±2.53

* - differences from the control are statistically significant at $p \leq 0.01$

of 16 hours the number of survived plants amounted to 2.9%, which is 63.1% less than in the control. Treatment for 6 hours was also effective. At the same mutagen concentration the survival rate of plants in this case was 49.4% lower than in the control and decreased to 16.6 ±1.99%.

The action of ethyl methanesulfonate was significantly weaker. In our study this mutagen had the most significant effect when seeds were treated with 0.05% solution at the exposure of 16 hours and with 0.1% solution at the exposure of 6 hours. In this case about 43-47% of the plants survived. In other treatments EMS had an even slighter effect on nigella plants, although its influence was statistically significant and differed from the control (when treated with distilled water the survival rate of plants reached 66.0%). Treatment with the new mutagen DG-2 had the weakest effect on the survival of M1 plants. Under the action of this substance, the portion of surviving plants was 4.9-12.9% lower than in the control. The most significant effect was observed in the variant with DG-2 concentration of 0.05% and the exposure of 16 hours, where the plant survival was at the level of 53.1 ± 2.67%.

In the M_1 generation, 10 plants with a change in the color and shape of leaves were found. Among plants with morphoses chlorophyll chimeras with a change in the color of leaves and calyx leaves, as well as plants with a change in the shape of leaves and dwarf plants (Fig.) were found. Most morphoses were observed after treatment with nitrosomethylurea. After treatment with this mutagen at the concentration of 0.01% and 6 hours exposure, the number of plants with morphoses reached 3.87%. Chlorophilic morphoses of the leaves were also revealed. After treatment with NMU at the concentration of 0.05% and 6 hours exposure, the number of plants with morphoses was 3.45%. Here chlorophilic morphoses of leaves and calyx leaves were found as well. After treatment with nitrosomethylurea at the concentration of 0.01% and 16 hours exposure, the number of plants with morphoses was 0.96%. Another mutagen

that caused the appearance of morphoses was DG-2. After treatment with this substance at the concentration of 0.5% with the exposure of 16 hours, 0.48% of plants with chlorosis, leaf shape morphosis and dwarf plants were revealed. Among the control plants no specimens with changes in color and habitus were found.

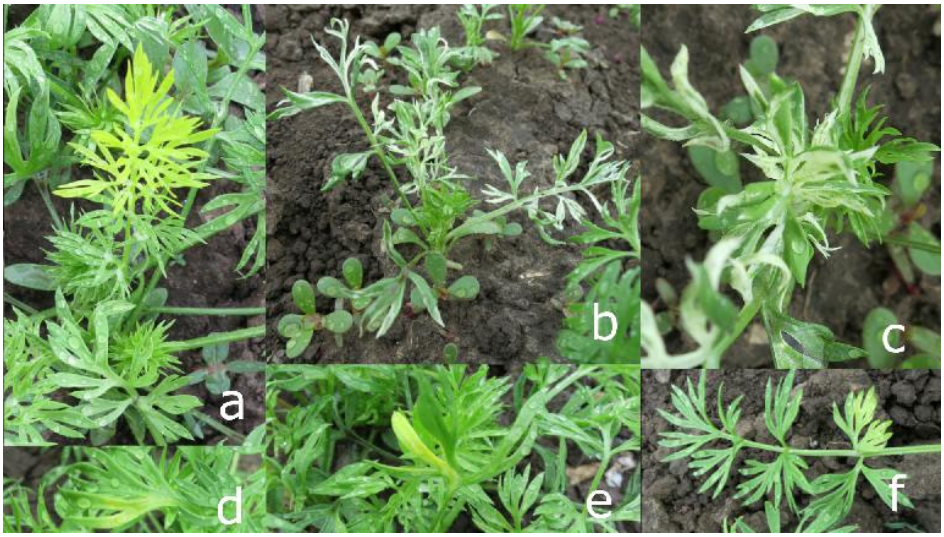

Fig. **Morphoses of plants of *Nigella damascena* L. variety Bereginya in generation M_1: *a, d-f - chlorophyll morphoses of leaves, b-c - chloroses and leaf shape morphoses.***

The presence of morphoses in the experimental M_1 generations and their absence in the control variants indicates the effectiveness of the action of mutagens and the possibility of obtaining mutant changes in the object of study in the future.

An important criterion for the effectiveness of the action of mutagens is the change in height and other morphometric parameters of plants in the first mutant generation. As can be seen from Table 2, all chemical mutagens led to a decrease in plant survival rate and mainly caused plant depression. In all treatments, a decrease in the total plant height was revealed in comparison with the control. Thus, the height of plants of the Bereginya variety decreased from 39.8 cm when treated with DG-2 at the concentration of 0.5% with 16 hours exposure to 21.2 cm when treated with nitrosomethylurea at the concentration of 0.05% and 6 hours exposure . In addition, in a number of cases a decrease in the number of seeds per plant was noted. In particular, treatment with nitrosomethylurea at the concentration of 0.05% and 16 hours exposure resulted in a decreasing seed set to 98.3, at the concentration of 0.01% and 16 hours exposure – to 77.4, and at the concentration of 0.01% and 6 hours exposure - to 48.2 seeds per plant.

In the variants where the seeds were treated with nitrosomethylurea at the concentration of 0.01 and 0.05% with 16 hours exposure, an increase in the number of lateral shoots and the length of the largest lateral shoot in relation to the control group

Table 2

Changes in a number of morphometric characteristics of plants of *Nigella damascena* L. variety Bereginya in generation M_1 as a result of seed treatment with chemical mutagens

Mutagen	Concentration, %	Exposition, hour.	Heigh, cm	The length of the largest shoot, cm	Number of side shoots, pieces	Number of capsules, pieces	Number of seeds, pieces
EMS	0.01	6	36.4±1.45*	8.3±1.64	4.1±0.74	4.6±0.65	133.1±26.02**
EMS	0.01	16	35.0±1.07*	9.1±1.55	4.5±0.70	5.2±0.57	150.3±27.70
EMS	0.05	6	34.7±1.73*	8.2±1.86	3.4±0.71	4.4±0.71	143.0±31.68
EMS	0.05	16	36.0±1.04*	11.3±1.76	4.9±0.66	5.9±0.65	191.7±39.58
NMU	0.01	6	25.0±1.40*	7.6±1.71	3.7±0.80	4.8±0.97	48.2±22.01*
NMU	0.01	16	22.5±1.17*	12.2±1.45*	7.7±0.95*	9.6±1.63*	77.4±28.11*
NMU	0.05	6	21.2±1.12*	9.8±1.95	7.4±1.80	7.9±1.84	134.8±62.02
NMU	0.05	16	21.5±1.57*	11.6±1.32*	6.3±0.90*	9.7±2.24*	98.3±30.14*
DG-2	0.05	6	36.6±1.19*	9.5±1.75	4.5±0.69	5.1±0.61	159.6±30.87
DG-2	0.05	16	38.9±1.17*	9.1±1.61	3.7±0.62	4.7±0.63	169.6±32.20
DG-2	0.5	6	34.8±1.02*	8.7±1.47	4.6±0.69	5.3±0.71	158.0±43.29
DG-2	0.5	16	39.8±0,92**	11.9±2.07	5.0±0.80	5.9±0.80	208.9±53.54
Distilled water		16	42.6±0.79	9.1±1.83	4.2±0.88	4.7±0.68	204.6±24.12

***, **, * - differences from the control are statistically significant at $p \leq 0.01$, 0.05 and 0.1, respectively.

was noted. However, this type of treatment most strongly, in comparison with other mutagens, reduced the number of surviving plants – down to 2.9%, that caused a rarer arrangement of plants on the experimental plot and, hence, providing a larger area for their nutrition.

Among the mutagens we used, nitrosomethylurea had imposed the greatest effect on plant height and number of seeds per plant, regardless of concentration and exposure used. Thus, the average number of seeds per plant turned out to be minimal after treatment with this substance at the concentration of 0.01% and 6 hours exposure – in this case it came to 48.2 seeds. Plant height decreased to the minimum value – 21.2 cm, after treatment with this mutagen at the concentration of 0.05% and 6 hours exposure.

Treatment with ethyl methanesulfonate induced much less changes in the characteristics studied. Among the variants where this substance was used as a mutagen, the most noticeable effect on plant height had the treatment of seeds with EMS at the concentration of 0.05% and 6 hours exposure, causing a decrease in plant height to 34.7 cm. The most significant negative effect on the number of seeds per plant was exerted by treatment with this substance at the concentration of 0.05% during 6 hours exposure, in that case the number of seeds amounted to 143. The control values after treatment of seeds with distilled water were equal to 42.6 cm and 204.6 seeds, accordingly.

The smallest, although statistically significant, effect on the height of plants of the M_1 generation in our study was exerted by the DG-2 mutagen. After treatment of seeds with this substance at the concentration of 0.5% and 6 hours exposure, the average plant height decreased down to 34.8 cm. In other treatments with DG-2 similar changes were observed. So, at the concentration of 0.05% and 6 hours exposure the average plant height was 36.6 cm, and the average number of seeds amounted to 159.6. Similar to our study, in the M_1 generation of brown mustard, when the seeds were treated with ethyl methanesulfonate at concentrations of 0.1–0.5%, a depression of recorded characteristics was observed (Lyakh et al, 2009). In wheat in the M_1 generation, the inhibitory effect of high doses and concentrations of mutagens on plant height and the number of seeds per plant was noted as well (Oks'om, 2010). At the same time, in wild flax, no decrease in plant height was observed after the action of EMS, except for the maximum concentration of 0.5%, which had an inhibitory effect on plant height (Lagron, Lyakh, 2000). In the same work, the inhibitory effect of ethyl methanesulfonate on the number of capsules in flax was shown, which we did not reveal in nigella.

A number of researchers note the stimulating effect of low concentrations of chemical mutagens on the traits of plants in the first mutant generation, for example, in flax and wheat (Tigova, Soroka, 2016; Oks'om, 2010).

In wheat, for example, in the M_1 generation, a stimulating effect of low doses and concentrations of mutagens on plant height and the number of spikelets in the main spike was observed (Oks'om, 2010). However, in our study with nigella no changes in this direction were found. We recorded only a tendency towards an increase of such traits as length of the largest lateral shoot and the number of lateral shoot.

Conclusions

Nitrosomethylurea had the greatest influence on the survival of plants of the *Nigella damascena* L. variety Bereginya in the M_1 generation. The number of plants

that survived after seed treatment with this substance, depending on the concentration and exposure, decreased in comparison with the control by 4.8-63.1%.

Treatment with the tested mutagens led to the appearance of chimeras with a frequency of 3.87% to 0.48%, characterized by a change in color and shape of leaves and the dwarf plants appearance.

All mutagens in our study, except for DG-2, had a depressing effect on the "plant height" trait. The most significant influence was exerted by nitrosomethylurea. The weakest influence among the studied mutagens was inherent to the new mutagen DG-2.

In general, the studied chemical mutagens exhibited a general biological effect on nigella plants in the M_1 generation, causing a delay in plant growth and development.

Change of many studied characteristics in M1 nigella plants after mutagenic treatment implies high rate of mutations in the following generations.

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ВПЛИВ ХИМИЧНИХ МУТАГЕНІВ НА ПОКАЗНИКИ РОСЛИН *NIGELLA DAMASCENA* L. СОРТУ БЕРЕГИНЯ ПОКОЛІННЯ M_1

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В роботі вивчено вплив хімічних мутагенів ЕМС, НМС і нового мутагена ДГ-2 на ряд ознак *Nigella damascena* L. сорту Берегіня в поколінні M_1. Встановлено, що обробка насіння чорнушки дамаської даними хімічними сполуками змінювала такі характеристики як виживання рослин, висота рослини і кількість насіння з однієї рослини, кількість коробочок на рослині та кількість бічних пагонів. Встановлено залежність зміни вивчених ознак від виду хімічного мутагену, його концентрації та тривалості експозиції. Найбільший вплив на виживання рослин справила нітрозометилсечовина. Після обробки даним мутагеном в максимальній концентрації 0,05% і при максимальній експозиції 16 годин кількість рослин, що вижили, склала 2,9%. Найменше на виживання рослин M_1 вплинув, порівняно з контролем мутаген ДГ-2 в концентрації 0,5% з експозицією 6 годин. В даному варіанті вижило 61,1% рослин, при контрольному значенні 66,0%. Випробувані мутагени викликали у рослин появу хлорофільних морфозів з частотою до 3.87%, вони характеризувались зміною кольору листя і листочків чашечки та карликовістю рослин. Найбільший вплив на висоту рослин і кількість насіння з однієї рослини справила нітрозометилсечовина в концентраціях 0,01 та 0,05% з експозицією 6 і 16 годин. Після обробки НМС в концентрації 0,01% з експозицією 6 годин, середня кількість насіння з однієї рослини була мінімальною – 48,2 штук, а висота рослин - 25,0 см. Найменший, але статистично значимий вплив на вивчені показники рослин надав мутаген ДГ-2. Після обробки ним в концентрації 0,05% з експозицією 16 годин середня висота рослин склала 38,9 см, а середня кількість насіння – 169,6 штук, при контрольних значеннях 42,6% та 204,6 шт. Виявлені зміни у поколінні M_1 передбачають отримання в наступному поколінні чорнушки дамаської мутацій різної спрямованості.

Ключові слова: *Nigella damascena*, чорнушка, мутагенез, покоління M_1, ЕМС, НМС, ДГ-2 морфоз, виживання, морфометрична характеристика.

ВЛИЯНИЕ ХИМИЧЕСКИХ МУТАГЕНОВ НА ПОКАЗАТЕЛИ РАСТЕНИЙ *NIGELLA DAMASCENA* L. СОРТА БЕРЕГИНЯ ПОКОЛЕНИЯ M_1

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В роботе изучено влияние химических мутагенов ЭМС, НММ и нового мутагена ДГ-2 на ряд показателей *Nigella damascena* L. сорта Берегиня в поколении M_1. Установлено, что обработка семян чернушки дамасской данными химическими веществами изменяла такие характеристики, как выживание растений, высота растений и количество семян с одного растения, также было отмечено изменение количества коробочек на растении и количество боковых побегов. Установлена зависимость изменения изученных признаков от вида химического мутагена, его концентрации и длительности экспозиции. Наибольшее влияние на выживание растений оказала нитрозометилмочевина. После обработки

данным мутагеном с максимальной концентрацией 0,05 % и при максимальной экспозиции 16 часов, количество выживших растений составило 2,9 %. Меньше всего на выживание растений M_1 повлиял, по сравнению с контролем, мутаген ДГ-2 с концентрацией 0,5 % при экспозиции 6 часов. В данном варианте выжило 61,1 % растений, при контрольном значении 66,0 %. Испытанные мутагены вызывали у растений появление хлорофильных морфозов с частотой до 3,87 %, они характеризовались изменением цвета листьев и листочков чашечки, и карликовостью растений Наибольшее влияние на высоту растений и количество семян с одного растения оказала нитрозометилмочевина в концентрации 0,01 и 0,05 % с экспозицией 6 и16 часов. После обработки НММ в концентрации 0,01 % с экспозицией 6 часов, среднее количество семян с одного растения было минимальным – 48,2 штук, а высота растений – 25,0 см. Наименьшее, но статистически значимое влияние на изученные показатели растений оказал мутаген ДГ-2. После обработки им в концентрации 0,05 % с экспозицией 16 часов, средняя высота растений составила 38,9 см, а среднее количество семян – 169,6 штук, при контрольных значениях 42,6 % и 204,6 шт. Выявленные изменения в поколении M_1 предусматривают получение в последующем поколении чернушки дамасской мутации разной направленности.

Ключевые слова: *Nigella damascena,* чернушка, мутагенез, поколение M_1, ЭМС, НММ, ДГ-2 морфоз, выживаемость, морфометрическая характеристика.