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Use of new derivatives of dimethyl sulphate for deriving hereditary variations at oil flux

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The purpose. To determine efficiency of induction of mutations of oil flux by means of new chemical mutagens, derivatives of dimethyl sulphate — DG-2, DG-6, DG-7, DG-9, to establish directedness of their action in comparison with initial substance and to create on this basis of new initial stock for selection. **Methods.** Field, laboratory, mathematical-statistical. **Results.** It is established that owing to treatment of seeds of flux of two varieties by new chemical mutagens DG-2, DG-6, DG-7, and DG-9 in densities of 0,5 and 0,05 % the wide spectrum of mutations (29 types) is gained in generation $_{M2}$. They were divided into 6 groups: 4 groups with changes of morphological type, one group with changes of physiological type, and one — with biochemical type. As a result the collection is gained of mutant samples which are donors of marker and economic-valuable attributes, and also different on biochemical contents of oil. **Conclusions.** For induction of mutations with breaking synthesis of chlorophyll pigment the most effective was mutagen DG-9, for mutations of structure of stalk, shoots and leaves — mutagens DG-7 (for variety Iceberg) and DG-6 (for variety Solnechnyi), for mutations of flower — DG-2, and also DG-9 (for variety Iceberg) and DG-7 (for variety Solnechnyi), for mutations of colour of seeds at variety Iceberg — mutagens DG-2 and DG-9, at variety Solnechnyi — mutagens DG-2 and DG-7, and for mutations to physiological attributes of growth and development — mutagen DG-2. The most effective at change of biochemical indexes of oil in seeds was mutagen DG-2.

Key words: *flux, mutagenesis, chemical mutagen, dimethyl sulphate, mutation, content of fat oils.*

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Mutations are the basis of the hereditary variability of all living organisms. Any changes in the genetic material can be induced by physical (ultraviolet radiation, short-wave radiation, etc.), biological (viruses, bacteria) and chemical mutagens at a much greater frequency than spontaneous mutations [1-4].

The method of induced mutagenesis makes it possible to obtain valuable breeding material with inherited properties and characteristics. According to J.A. Rapoport, creation of mutant varieties is based on the high frequency of new beneficial mutations that mobilize attributes not available to other breeding methods. With the help of experimental mutagenesis, it is possible to make the hidden part of the mutational spectrum work, getting a stable genetic state with new valuable properties and traits [5].

The advantage of the method of experimental mutagenesis, compared with the methods of hybridization is a shorter term for which you can obtain valuable breeding material. This makes it possible to raise varieties almost twice as fast. However, works on mutation selection are carried out on a limited scale, yielding in volume from 50 to 500 times less than the selection based on hybridization [1, 5].

Nevertheless, to date, with the help of the method of experimental mutagenesis, about 3218 mutant varieties of various crops have been created, including grain, oilseed, leguminous, vegetable, fruit and ornamental plants. Cultivating mutant varieties of various crops has significantly improved the level of food security, which has led to an increase in the number of food products worldwide [6, 7].

One of the crops that is becoming increasingly important is oil flax. This is a valuable, non-waste crop, which is used in various branches of the national economy and medicine. Cultivation of oil flax is stipulated by its significant economic efficiency. Due to a high oil content (45-50 %) and potential yield (2.0-2.5 t/ha), it is very interesting for producers. The development of the flax industry in modern conditions is impossible without the production of high-quality and competitive products based on, first of all, high yields. One of the main factors in increasing a flax seed yield is the development and introduction into production of new high-yielding varieties with marker and original characteristics that would meet the requirements of the producers and processors.

Successful solving problems for obtaining such material is possible with the use of highly effective methods, in particular the method of induced mutagenesis. This method allows in a short time to create a new source material with a variety of morphological and physiological characteristics, biochemical parameters, to increase the frequency and expand the range of original mutations. At the same time, side by side with the use of classical chemical mutagens, investigations of new compounds capable to induce mutations are proceeded, as there is a need to raise certain changes with a higher frequency. In addition, the need to protect the environment determines a search for substances with a high mutagenic effect and less toxicity. One of such substances could be derivatives of dimethyl sulfate (DMS) – a chemical mutagen, which has already proved its effectiveness in previous studies for a number of species. However, the effect of DMS on flax has been poorly studied, and various derivatives of this substance have not been analyzed at all. In this regard, the study of the effectiveness of the action of new chemical mutagens, synthesized on the basis of DMS (DG series), are extremely important and relevant.

The purpose. The research is aimed to determine the effectiveness of inducing mutations in oil flax with the help of new chemical mutagens DG-2, DG-6, DG-7, and DG-9, and to determine the direction of their action in comparison with the parent compound (the DMS mutagen), and to develop on this basis a new source material for breeding.

Materials and methods of research. Experiments were carried out during 2014-2018 at the Institute of Oilseed Crops of the National Academy of Agrarian Sciences (IOC NAAS) of the Ukraine with two oil flax varieties of *Linum humile* Mill., – Iceberg and Solnechny. Iceberg variety was created at the IOC NAAS. It has white petals, star-shaped flowers. Seeds are dark brown, 1000 seed weight is 7.7 g. It is characterized by high iodine number of oil (17.6), resistance to drought, lodging, Fusarium wilt. Since 2001 it has been included in the State Register of Plant Varieties of the Ukraine [8].

Solnechny variety was created at the Flax Institute (Belarus). Flowers are of blue color with a violet shade, seeds - of yellow color. 1000 seed weight is from 5.2 to 5.5 g. It is characterized by a specific fatty acid composition with a reduced to 10 % content of linolenic acid [9, 10].

Mutagens of the DG series (DG-2, DG-6, DG-7, and DG-9) are new chemical compounds, derivatives of dimethyl sulfate, synthesized at the Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of the Ukraine and given to us by Ph.D. Dulnev. The essence of the mutagenic action of DMS is the reaction of alkylation of the DNA molecule by the incorporation of ethyl or methyl groups. It is also known from the scientific literature that DMS often causes chromosomal ruptures and that most of the reunions are intrachromosome, which leads to the formation of a large number of chromosomal inversions [11-14].

The air-dry seeds of two flax varieties were treated with aqueous solutions of the chemical mutagens DG-2, DG-6, DG-7, DG-9, and DMS at the concentrations of 0.05 and 0.5 % for 16 hours. For each treatment we used 300 seeds in one repetition. In control (K) seeds of the appropriate varieties were soaked in distilled water. After treatment the seeds were washed for one hour under running water, and on the same day they were sown in the field to get the M_1 generation. In the literature there are data on a decrease in mutagenic damage when seeds are washed under water, with a lethality and sterility dramatically reduced at a constant mutability [15].

From the individual M_1 plants seeds were harvested and sowed next year to produce the M_2 generation. In this generation mutations of the morphological and physiological types were searched for at different stages of development, and those mutant plants were subsequently evaluated according to biochemical parameters. The final frequency of mutations was counted after confirmation of their inheritance in the next generation of M_3. The frequency of mutations was calculated as the ratio of the number of mutant families to their total number. The seed oil content was determined by the weight of the fat-free residue in the Soxhlet apparatus and the fatty acid composition of the oil – by the gas-liquid chromatography of the methyl esters on the Hewlett Packard HP 6890 chromatographer.

Research results. Treatment of flax seeds with the new chemical mutagens DG-2, DG-6, DG-7, DG-9 at the concentrations of 0.5 and 0.05 % has led to the generation of a broad spectrum of mutations in the M_2 generation, which was represented by 29 types of changes that were subdivided into 6 groups – 4 groups with changes of morphological type and one group with changes of physiological and of biochemical types (Table 1-3):

I. Mutations with chlorophyll-deficiecy (8 types): *albina, viridis-albina, xantha, chlorina, viridis, lutescent, striata, corroded*.

II. Mutations in the structure of stem, shoots and leaves (5 types): *three cotyledons, tall plants, small plants, dwarfs, zigzag-shaped stem.*

III. Mutations in the color of corolla petals and pollen, the shape of the flower and flower bud (6 types): *light-blue petals, creamy anthers; blue petals, blue anthers; modified shape of petals; pale pink petals, creamy anthers; white petals, creamy anthers; a corolla that is not opened*.

IV. Mutations of seed colour (4 types): *yellow, brown, mustard, mottled*.

V. Mutations for physiological traits of growth and development (4 types): *early-ripening plants, late-ripening plants, sterile plants, disturbance of seed development*.

VI. Mutations in biochemical characteristics (2 types): *oil content, fatty acid composition of oil*.

Table 1. The overall frequency of induced morphological mutations in *Linum humile* Mill. in the generation M_2, % (2016-2018)

Total families / Type of mutation *	Mutagen (0.5 & 0.05 %)					
	К	**DG-2**	**DG-6**	**DG-7**	**DG-9**	**DMS**
	Iceberg variety					
	100	210	107	209	219	106
I	0.00	2.85±1.15	1.96±1.34	7.46±1.82	10.95±2.11	0.00
II	0.00	3.81±1.32	0.00	3.83±1.33	1.82±0.90	0.00
III	0.00	6.68±1.72	0.00	1.98±0.96	3.65±1.27	0.00
IV	0.00	2.88±1.15	0.00	1.98±0.96	2.74±1.10	0.00
Total mutations, %	0.00	16.22±2.54	1.96±1.34	15.25±2.49	19.16±2.66	0.00
Solnechny variety						
Total families / Type of mutation	100	205	124	202	206	105
I	0.00	10.76±2.16	9.70±2.66	10.89±2.19	18.43±2.70	0.95±0.95
II	0.00	0.00	2.91±1.51	1.98±0.98	0.00	0.00
III	0.00	10.72±2.16	1.94±1.24	6.93±1.79	3.88±1.34	1.90±1.33
IV	0.00	10.70±2.16	5.82±2.10	5.94±1.66	2.91±1.17	0.95±0.95
Total mutations, %	0.00	32.18±3.26	20.37±3.62	25.74±3.08	25.22±3.02	2.85±1.62

* Note: I – mutations with chlorophyll-deficiency; II – mutations in the structure of stem, shoots and leaves; III – mutations in the color of corolla petals and pollen, the shape of the flower and flower bud; IV – mutations of seed colour.

Mutagens of the DG-series, as well as the classical mutagen DMS, caused the appearance of hereditary changes of morphological nature with different frequency in different groups. The effectiveness of each mutagen is directly proportional to the frequency of mutations that was induced by it. However, the compounds studied significantly differed in the spectrum of mutations they caused. Thus, the mutagen DG-2 was found to be the most effective for obtaining a group of flower mutations, which included the changes in the color of corolla and pollen, the shape of petals and flower buds, both for the Solnechny and Iceberg varieties. Among all the mutagens studied, this mutagen initiated a maximum frequency of mutations for changing the color of corolla and anthers. It was 6.68 % for the Iceberg variety and 10.72 % for the Solnechny variety, while the mutation frequency caused by the classical DMS mutagen in this group was significantly lower and equaled to 1.90 % for the Solnechny variety, while for the Iceberg variety mutations were not detected at all (Table 1). In addition to mutations of the flower, the mutagen DG-2 was effective for the induction of mutations of other types, proving the effectiveness of its usage.

The mutagen DG-6 was characterized by a narrow spectrum of action. It was effective in the induction of chlorophyll-deficient changes. In the Iceberg variety it caused 1.96 % of mutations and only of this type, and in the Solnechny variety disorder of chlorophyll synthesis was observed in 9.70 % of families. In the variety Solnechny the given mutagen caused other changes as well. For instance, it was more effective than the source compound when mutations were induced in the structure of the stem, leaves and seed color.

The mutagen DG-7 had a wide range of effects, since in both varieties it stimulated the appearance of all studied types of mutations. The most effective for the Iceberg and Solnechny varieties the mutagen DG-7 was to induce mutations with a violation of the chlorophyll synthesis, which amounted to 7.46 and 10.89 %, respectively. The frequency of other types of changes was also high enough and exceeded the original substance.

The mutagen DG-9 was also characterized by a wide range of effects. Like DG-7, it caused mutations with a violation of chlorophyll synthesis in both studied varieties with a maximum frequency of 10.95 % for the Iceberg variety and 18.43 % for the Solnechny variety. Changes of other types the compound DG-9 initiated in a less amount but their frequency was still higher than the frequency of changes induced by the source DMS substance. The mutagen DG-9 was ineffective only to induce mutations in the structure of the stem, shoots and leaves in the Solnechny variety.

The mutagen DMS proved to be ineffective in comparison with the new chemical mutagens of the DG-series synthesized on its basis. In the Iceberg variety it did not cause any type of morphological changes. In the Solnechny variety this mutagen was also ineffective in comparison with the new chemical mutagens.

Table 2. The overall frequency of physiological mutations induced in *Linum humile* Mill. in the generation M_2, % (2016-2018)

Total families / Type of mutation	Mutagen (0.5 & 0.05 %)					
	K	**DG-2**	**DG-6**	**DG-7**	**DG-9**	**DMS**
	Iceberg variety					
	100	210	107	209	219	106
Early-ripening plants	0.00	0.96±0.67	0.00	0.99±0.68	0.92±0.64	0.00
Late-ripening plants	0.00	0.94±0.66	0.00	0.00	0.00	0.94±0.94
Sterile plants	0.00	0.00	0.00	0.00	0.00	0.00
Total mutations, %	0.00	1.90±0.94	0.00	0.99±0.68	0.92±0.64	0.94±0.94
Solnechny variety						
Total families / Type of mutation	100	205	124	202	206	105
Early-ripening plants	0.00	0.98±0.69	0.00	0.99± 0.70	0.00	0.00
Late-ripening plants	0.00	0.97±0.68	0.00	0.99±0.70	0.00	0.95±0.95
Sterile plants	0.00	0.97±0.68	0.00	0.00	0.00	0.00
Total mutations,%	0.00	2.92±1.18	0.00	1.98±0.98	0.00	0.95±0.95

Regarding the mutations for physiological traits of growth and development, the mutagen DG-2 appeared to be effective, stimulating in this case the appearance of early and late-ripening plants in the Iceberg variety with a frequency of 0.96 and 0.94 %, respectively (Table 2). In the Solnechny variety this compound caused the emergence of sterile plants at a frequency of 0.97 %.

The mutagen DG-6 proved to be ineffective in terms of stimulating physiological mutations, which suggests the inexpediency of its use in this direction.

Treatment with the mutagen DG-7 stimulated the appearance of early ripening plants in both studied varieties with the same frequency of 0.99 %. In addition, this compound in the Solnechny variety caused the appearance of plants with a later date of ripening with the same frequency.

The mutagen DG-9 proved to be effective only for the Iceberg variety at the induction of early-ripening plants.

The parent compound – DMS, speaking of induction of mutations in physiological traits of growth and development was effective for late-ripening plants and caused their appearance at a frequency of 0.94–0.95 % in both studied varieties.

Thus, the mutagen DG-2 was the most effective for induction of physiological mutations of growth and development, although other tested chemical mutagens demonstrated a positive effect as well.

In addition to the above changes, DMS and its derivatives initiated mutations at the biochemical level. For example, if the total oil content of the Iceberg seeds in control was 47.23 %, then the mutants obtained after the treatment with the DG-7 and DG-9 mutagens exceeded the control for this parameter (Table 3). When treated with the mutagen DG-2, the greatest variability was found, which reached 35.15 %, compared to other mutagens. This suggests significant opportunities for using this mutagen to influence the studied traits. The total oil content in the control of the Solnechny variety was 40.69 %. Treatment with the mutagens in all cases has led to the origin of mutants that exceeded the control for this characteristic. The largest range of variability was found in the treatment with the mutagens DG-2 and DG-9, it amounted to 5.99 and 5.05 %, respectively.

Table 3. The spin of variation in the *Linum humile* Mill. mutants, according to biochemical parameters in the generation M_2, % (2016-2018)

Mutagen (0,5 & 0,05 %) / **Biochemical parameters**	**К**	**DG-2** (min-max*)	**DG-6** (min-max)	**DG-7** (min-max)	**DG-9** (min-max)	**DMS** (min-max)
Iceberg variety						
Oil content	47.23	6.97-42.12	45.28-47.09	44.69-47.75	44.08-48.88	39.46-44.51
Palmitic acid (C16:0)	6.16	5.90-6.96	6.18-6.54	5.06-6.81	5.34-6.59	6.02-6.76
Stearic acid (C18:0)	3.71	1.63-4.13	2.39-6.54	1.36-4.15	1.93-3.29	2.47-4.48
Oleic acid (C18:1)	19.54	16.67-18.84	16.36-17.95	15.11-20.39	15.61-18.22	15.87-20.33
Linoleic acid (C18:2)	19.27	12.03-35.15	11.02-17.72	12.05-25.75	14.40-32.00	44.12-57.41
Linolenic acid (C18:3)	51.31	39.63-60.77	55.51-63.56	49.68-63.02	43.45-59.64	3.06-26.25
Solnechny variety						
Oil content	40.69	39.66-45.65	45.28-47.09	41.18-44.63	39.46-44.51	43.91-46.69
Palmitic acid (C16:0)	6.05	5.94-6.92	6.18-6.54	6.24-6.86	6.02-6.76	6.44-6.88
Stearic acid (C18:0)	5.81	1.19-3.93	2.39-6.54	1.95-4.10	2.47-4.48	1.71-2.52
Oleic acid (C18:1)	20.03	16.20-20.82	16.36-17.95	16.67-22.34	15.87-20.33	16.04-19.68
Linoleic acid (C18:2)	66.53	11.72-65.25	11.02-17.72	18.77-68.32	44.12-57.41	23.06-42.62
Linolenic acid (C18:3)	1.59	6.71-60.74	55.51-63.56	4.24-46.22	3.06-26.25	29.19-54.57

Note: * min-max is the minimum and maximum value of the parameters in the mutants studied

Regarding fatty acid composition of seed oil of both flax varieties, it is worth noting that the new mutagenic compounds caused significant changes in the ratio of all major fatty acids. Their content varied both at the direction of increase and reduction, which makes it possible to pick mutants of a diverse range of uses. For example, in the case of palmitic acid, the range of variability of its content in the oil varied from 0.36 to 1.75 %, with the lowest level of 5.06 %, and the highest - 6.96 %, while the control value was 6.05-6.16 %. The sharpest shift in the content of palmitic acid in the Iceberg and Solnechny varieties was caused with the mutagens DG-7 and DG-2 respectively.

Unlike palmitic acid, treatment with the new chemical mutagens often resulted in decrease in stearic acid levels as relative to the control. So, among the mutants of the Solnechny variety, there were no highly stearic specimens, and the lowest level of this acid (only 1.19 %) was detected in the treatment with the mutagen DG-2. For the Iceberg variety the situation turned to be different. All mutagens, except

DG-9, caused the change of this parameter not only in the direction of reduction but also in the direction of increase. The maximum magnitude of variation of 4.15 % was characteristic for the DG-6 compound, which induced an increase in the stearic acid content to 6.54 %.

The content of oleic acid in most of the mutants obtained was lower or at the level of the control. Mutants with an elevated level of this acid in both varieties were found in the treatment with the mutagen DG-7. In addition, this mutagen also caused the largest range of variability in the content of this acid. In treatments where the source mutagen DMS was used there were not found mutant samples, which exceeded the control for the content of oleic acid.

The level of variation for linoleic acid among the studied mutant accessions significantly depended on the variety. In the genotype with a high initial content of this acid mutations mainly decreased its amount. For the Iceberg variety, where linoleic acid amounted to 19.27 %, the new tested mutagens induced changes of different directions. Thus, the tested mutants had a content of linoleic acid of 11.02 to 35.15 %. The largest range of variability in both genotypes was caused with the mutagen DG-2 – from 23.1 to 53.5 %. It is this substance that should be used in breeding work aimed at increasing the genetic diversity for linoleic acid.

As the content of linolenic acid correlates with the content of linoleic acid, in the studied mutants of flax with a reduced level of linoleic acid the treatment with chemical mutagens has led to an increase in the amount of linolenic acid. This is especially noticeable for the mutants of the Solnechny variety, where the initial content of this acid was less than 2 %. Obviously, the tested mutagens caused reverse mutation in one of the *FAD3* gene loci, which, according to the literature, are responsible for the synthesis of desaturases, initiating the third double bond in the linoleic acid molecule are transforming it into linolenic. The greatest range of variation for linolenic acid was observed in the treatment with the mutagen DG-2, it was 21.14 % (in the Iceberg variety) and 54.03 % (in the Solnechny variety), with the minimum value for this indicator at 7 %, and the maximum – at about 61%.

Conclusions

Thus, in this paper the effectiveness of the use of new chemical mutagens, dimethyl sulphate derivatives – DG-2, DG-6, DG-7, DG-9 is shown to get a wide spectrum of mutant lines and specimens by morphological, physiological and biochemical characteristics. It was found that the Iceberg variety was characterized by a narrow spectrum of morpho-physiological changes (16 types) compared to the Solnechny variety (22 types). It was shown that the frequency of morphological and physiological mutations in the generation of M_2 was quite high and depended on genotype and concentration of the mutagen. The frequency of morphological mutations was 1.96-16.22 % (Iceberg variety) and 20.37-32.18 % (Solnechny variety), physiological – 0.92-1.90% (Iceberg variety) and 1.98-2.92 % (Solnechny variety). The range of variability in the level of oil content and different fatty acids was quite broad (0.36-54.03 %) which allows to use new chemical mutagens in breeding work of different orientations.

The increase of genetic diversity for the given parameters permits to carry out scientific work and raise new varieties of oil flax with both new marker and economically valuable traits, for example, color of flower and seed coat, height of plants, duration of vegetation period, different levels of oil content and fatty acids.

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