Experimental mutagenesis in fiber flax breeding

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The effects of different concentrations of nitrosoethyl urea, nitrosomethyl urea, ethyl methanesulphonate, ethylene imine, dimethyl sulphonate and various doses of gamma rays on variability of the flax varieties 'Svetoch' and 'Vaižgantas' were tested. The frequency and spectrum of mutations as well as the number of useful mutant types were determined in generation M_2. In later generations the mutant lines, distinguishing themselves by valuable qualities, were selected and some were involved into breeding process.

Key words: fiber flax, mutagenesis, mutagen concentration, dose, mutant type

INTRODUCTION

Besides application of intervarietal crossing and selection in fiber flax and linseed breeding, methods of physical and chemical mutagenesis [1–4] are applied to create the source material. The application of these methods yielded many valuable flax mutants [5, 9] and varieties [10–12].

The following chemical mutagen are widely used in experimental mutagenesis: NEU (nitrosoethyl urea), NMU (nitrosomethyl urea), EMS (ethyl methanesulphonate), EI (ethylene imine), DMS (dimethyl sulphonate) [1, 6, 7, 13] and also physical mutagens – X-rays and gamma rays [13–15]. It has been established that, by treating linseeds with ionizing radiation or chemical agents, it is possible to obtain new plant forms characterized by a diversity of morphological characters or physiological properties [3, 5, 10, 16].

The aim of our investigations was to research the reaction of the fiber flax varieties 'Svetoch' and 'Vaižgantas' to the effect of various rates of gamma rays and concentrations of chemical mutagens, to investigate the mutability of these varieties, the frequency of inductive mutations and their dependence on a mutagen, its concentration or dose, the spectrum of mutations, as well as to determine the number of mutant types useful for fiber flax breeding, to select the flax mutant lines distinguishing themselves with valuable properties and characters.

MATERIALS AND METHODS

Seeds of the flax varieties 'Svetoch' and 'Vaižgantas' were irradiated with gamma rays (the source was ^{60}Co) in the Laboratory of Radiation biophysics at the Institute of Agrophysics (St. Petersburg) at the following 14 doses: 1; 5; 11,5; 20; 30; 40; 50; 60; 70; 80; 90; 100; 125 and 150 KR (KR – kiloroentgen, 1 KR = 1000 roentgens), and the gamma rays – 750 R/min. One thousand seeds were treated in each treatment. In the other trial seeds were soaked in water solutions of NEU – 0.012; 0.025 and 0.05% for 18 h; of NMU – 0.006; 0.012 and 0.025% for 18 h, 1000 seeds were treated in each treatment; of EMS – 0.1; 0.2; 0.3 and 0.4%; of EI – 0.01; 0.02; 0.03; 0.04 and 0.05% for 12 h and for 18 hrs soaked in water solution of DMS – 0.016; 0.025 and 0.05% (500 seeds). The seeds were treated with mutagen in accordance with the methodic instructions issued by the Institute of Chemical Physics at the Russian AS [17]. The soaked seeds were washed with water, then slightly dried and sown in the trial field within 2–3 days, while seeds treated with ionizating radiation were sown within 6 days. They were sown in a 1 m belt with 10 cm interrow distance and 150 seeds per row. Seed germination, plant growth and development, various morphological and physiological deviations were observed in M_1 generations (1971). M_2 generations (1972) were sown out in separate lines, in which the character of changes caused by various treatments, their frequency and the spectrum of chlorophyllic, morphological and physiological mutations were observed. The number and frequency of induced mutations, depending on the treatment were established by the following methods: a) percentage of lines with changed plants (relative to the total number of lines); b) percentage of changed plants (relative to the total number of plants) [17].

The mode of inheritance of changes was established in M_3 and M_4 generations. After propagation of seeds, valuable lines were sown in the breeding nursery (1973–1986 and 1987–2000), in which economic–biological properties and characters of mutants and various parameters of productivity were established.

All field trials, phenological observations and laboratory tests were conducted according to methodical instruction [18].

The meteorological conditions during the years of the test (1973–2000) were not similar. The years 1973, 1975, 1979, 1982, 1990, 1991, 1993, 1996 and 1997 were favorable for flax growing. The seed and stem yield was perfect in those years. Not favorable weather conditions were in 1976, 1980, 1983, 1985 and 1998 – the flax was lodged by the rains. In 1992 and 1994 dry summer reduced flax yield. The yield was not great in all of the years.

RESULTS AND DISCUSSION

When treating with low (1; 5; and 11.5 KR) and high (125–150 KR) doses, no chlorophyllic mutations were noticed in M_2 generation. With increasing the irradiation dose (from 20 KR), the number of chlorophyllic mutations in both varieties changed differently. Most of them occurred in 'Vaižgantas' (treated with 50 KR (2.39%) and in 'Svetoch' at 70 KR (1.42%). The distribution of chlorophyllic mutations in both varieties in the M_2 generation was as follows: *viridis* 33.3% to 55.8%, *xantha* 10.0 to 55.6%, *striata* 5.6 to 28.5% and *maculata* 2.6 to 16.7%. Morphological and physiological mutations were most frequent (0.97 to 2.44%) in the flax variety 'Svetoch', when the seed was treated with 50 to 150 KR doses, while in 'Vaižgantas' (1.30 to 2.76%) they were found when the seed was treated with 80 to 125 KR. The following mutant types were revealed: large-seeded, small-seeded, early, late, long, with increased fibre output, lodging resistant, disease resistant, productive, etc. Also the number of mutant types was revealed depending on gamma rays (Table).

The same chlorophyllic, morphological and physiological flax mutations were noted in the trials with chemical mutagen in M_2 generation. The data show the number of changed plants to be directly proportional to the concentrations of NEU, NMU, EMS, EI and DMS of mutagens. Among chemical mutagens, the larger of mutant types were induced by NEU, NMU and EMS, and the smaller by EI and DMS.

Various selected mutant M_2 plants had a different degree of stability in M_3 and M_4 generations. Flax inherited morphological and physiological changes in the later generations as follows: increased fiber output 67–88%, increased earliness 70–100%, increased lateness 89–100%, increased lodging resistance 60–80%, seed size 60–80%, etc. Many valuable mutant lines were selected and tested in various breeding nurseries and provocative (infections of flax diseases) backgrounds [18] over 1976–1986 and 1987–1998. From the research data rather valuable lines were obtained. Some of them are described below:

S-5-197 (20 KR) produces a linseed yield by 25% higher than the initial variety 'Svetoch'. The fiber output is by 3.5–4.4% higher, the growth period is by 3 days longer.

S-2-22 (90 KR) gives linseed yield than by 15% higher 'Svetoch'. Fiber output is by 3.0–4.5% higher, fiber quality is good. Flax plants grow by 15 cm longer and their growth period is by 5 days longer. They are less damaged by seedling blight (antrachnose – *Colletotrichum lini* M. et B.).

Table. **The number of changed plants and mutant types in flax M_2 generation when applying gamma rays or chemical mutagen**

Mutagenic treatment	Changed plants		The number of mutant types			
			total		valuable for breeding	
Dose KR, concentration %	'Svetoch'	'Vaižgantas'	'Svetoch'	'Vaižgantas'	'Svetoch'	'Vaižgantas'
1	2	3	4	5	6	7
Gamma rays						
Check	0.41 ± 0.41	0.52 ± 0.52	1	1	0	0
1	0.46 ± 0.21	0.16 ± 0.11	2	3	0	1
5	0.48 ± 0.12	0.41 ± 0.24	5	5	0	1
11.5	0.53 ± 0.15	0.39 ± 0.19	7	4	1	2
20	0.65 ± 0.16	0.60 ± 0.24	6	6	2	3
30	0.51 ± 0.14	0.53 ± 0.14	9	6	2	3
40	0.73 ± 0.21	0.67 ± 0.13	10	11	2	4

1	2	3	4	5	6	7
50	0.97 ± 0.21	0.88 ± 0.26	12	9	3	4
60	1.36 ± 0.23	0.69 ± 0.17	12	11	4	4
70	1.22 ± 0.22	0.80 ± 0.21	13	11	4	4
80	1.52 ± 0.34	1.33 ± 0.34	12	10	4	4
90	1.45 ± 0.23	1.51 ± 0.37	14	8	3	3
100	1.02 ± 0.22	1.30 ± 0.26	10	9	2	1
125	0.83 ± 0.59	2.76 ± 0.61	3	7	0	1
150	2.44 ± 2.41	0.45 ± 0.45	1	2	0	0
Chemical mutagen						
Check	0.07 ± 0.04	0.05 ± 0.03	1	1	0	0
NEU 0.012	1.70 ± 0.10	1.60 ± 0.10	22	22	7	6
NEU 0.025	3.30 ± 0.20	2.30 ± 0.10	27	25	9	7
NEU 0.05	5.90 ± 0.30	3.50 ± 0.30	28	27	6	5
Check	0.07 ± 0.04	0.05 ± 0.03	0	0	0	0
NMU 0.006	1.80 ± 0.10	2.40 ± 0.20	22	29	9	5
NMU 0.012	4.50 ± 0.20	3.80 ± 0.20	26	25	9	4
NMU 0.025	4.60 ± 0.60	5.60 ± 0.60	25	25	5	3
Check	0.03 ± 0.03	0.02 ± 0.02	1	1	0	0
EMS 0.1	1.40 ± 0.30	1.40 ± 0.20	16	14	4	4
EMS 0.2	2.10 ± 0.40	3.20 ± 0.40	17	17	7	4
EMS 0.3	3.40 ± 0.50	4.50 ± 0.60	21	22	6	5
EMS 0.4	4.40 ± 0.70	3.60 ± 0.70	20	20	4	3
Check	0.70 ± 0.30	0.30 ± 0.20	0	0	0	0
EI 0.01	2.30 ± 0.30	2.10 ± 0.30	11	15	2	1
EI 0.02	2.80 ± 0.30	2.00 ± 0.30	13	16	2	1
EI 0.03	3.70 ± 0.30	2.60 ± 0.30	17	16	3	4
EI 0.04	3.50 ± 0.40	2.90 ± 0.30	18	19	2	3
EI 0.05	4.10 ± 0.50	2.60 ± 0.30	14	17	1	2
Check	0.20 ± 0.10	0.20 ± 0.20	0	0	0	0
DMS 0.016	1.70 ± 0.40	0.90 ± 0.30	12	15	3	2
DMS 0.025	1.90 ± 0.30	1.80 ± 0.30	14	13	2	2
DMS 0.05	1.80 ± 0.30	2.00 ± 0.40	13	12	1	1

V-26-228 (30 KR) produced a stem and linseed yield by 9% higher. Fiber output is by 2.8–3.7% higher, fiber quality is good. Growth period is shorter by 3 days, the plants are less damaged by fusariose (*Fusarium spp.).*

E-3-310-5 (NEU – 0.025%) gives by 7–8% higher stem and linseed yield than does 'Vaižgantas'. Fiber output is by 3.1% higher, its quality is good. The flax is by 0.7 points more lodging-resistant, the stems are less damaged by fusariose (*Fusarium* spp.) and rust (*Melampsora lini* Desm.).

F-5-398-4 (NMU – 0.006%) is white-blossomed, grows by 20% of stems and 14% of linseed more than the initial variety 'Vaižgantas'. Fiber output is by 2.3% higher. The flax plants grow up long, are lodging-resistant and less affected by fungal diseases.

G-2-109-5 (EMS – 0.4%) gives stem and linseed yield by 22–25% higher than does the initial variety 'Svetoch'. Fiber output is by 2% higher, its quality is good – soft, flexible, strong. The plants grow by 10 cm longer, by 0.8 points are more lodging-resistant. The mutant is white-blossomed.

The mutant lines C-3-313-4 (NEU – 0.025%), E-2-17-3, E-2-17-7 (NEU – 0.012%), F-5-168-4 (NMU – 0.006%), M-5-5-4 (EMS – 0.4%) and other were charaterized by abundant stem, linseed and fiber yield.

Different weather conditions over research years gave an opportunity to select lodging-resistant flax mutant lines such as E-2-17-3, E-2-17-6, E-2-17-7, E-2-147-4 (NEU – 0.012%), F-5-345-1, F-5-398-3 (NMU – 0.006%), 4V-3 (EI – 0.03%), 2V-6 (EI – 0.01%), F-6-66-1 (NMU – 0.012%), M-2-18-2 (EMS – 0.1%), M-3-114-4 (EMS – 0.2%), G-4-44-1 (EMS – 0.3%), etc.

The following mutant lines have high fiber output: A-3-56-7 (NEU – 0.025%), E-4-4-430-2 (NEU – 0.05%), M-4-44-4 (EMS – 0.4%), E-2-17-5, E-2-62-2 (NEU – 0.012%), etc.

A-3-126-1, E-3-343-2 (NEU – 0.025%), F-5-406-1, F-5-406-5 (NMU – 0.006%), G-3-74-4 (EMS –

0.2%) and other lines have a short growth period. E-2-147-4 (NEU – 0.012%), F-7-397-2 (NMU – 0.025%), M4-107-7 (EMS – 0.3%) and other mutant lines have a long growth period.

The longest stems amongst the tested mutants belonged to the lines A-2-41-1, A-2-56-4 (NEU – 0.012%), A-3-2-5 (NEU – 0.025%), F-6-66-4 (NMU – 0.006%), G-5-145-2 (EMS – 0.3%) and other.

Large linseeds characteristic to the lines: A-2-38-6, E-2-17-7, E-2-17-6 (NEU – 0.012%), A-3-2-5, A-3-56-7, E-3-224-2 (NEU – 0.025%), F-7-30-1 (NMU – 0.025%), M-5-44-4 (EMS – 0.4%).

Resistance to fusaria (*Fusarium* spp.) of fiber flax mutants C-2-115-3 (NEU – 0.012%), F-5-113-3 (NMU – 0.006%), M2-107-4, M-2107-7 (EMS – 0.1%), etc.

Resistance to rust (*Melampsora lini* Desm.) of fiber flax mutants: C-4-121-1 (NEU 0.05%), E-3-56-2 (NEU – 0.025%), F-5-230-1 (NMU – 0.006%), M-4-129-1 (EMS – 0.3%), M-5-74-5(EMS – 0.4%), etc.

CONCLUSIONS

1. The spectrum and frequency of mutations in M_2 generation of the flax varieties ‘Svetoch’ and ‘Vaižgantas’ treated with various doses of physical or chemical mutagenes slightly different.

2. To obtain a more useful flax mutation, it is necessary to treat seeds with 20–90 KR doses of gamma rays. When treating with a chemical mutagen, it is necessary to use only weak or medium concentrations: NEU – 0.012 and 0.025%, NMU – 0.006 and 0.012%, EMS – 0.1; 0.2 and 0.3%, EI – 0.01; 0.02 and 0.03% and DMS – 0.016 and 0.025%.

3. The frequency of induced mutations, their spectrum and development of valuable forms revealed the efficiency of the test mutagens (in reducing sequence): NEU ~ NMU > EMS > gamma rays > EI ~ DMS.

4. When utilising chemical and physical mutagens in fiber flax breeding, new flax mutant lines valuable for cultivation were selected and introduced into breeding programs.

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PLUOŠTINIŲ LINŲ EKSPERIMENTINĖ MUTAGENEZĖ

S a n t r a u k a

Tirtas cheminių mutagenų nitrozoetilšlapalo, nitrozometilšlapalo, etilmetansulfonato, etilenimino ir dimetilsulfato įvairių koncentracijų ir fizinio mutageno – gama spindulių įvairių dozių įtaka veislių ‘Svetoč’ ir ‘Vaižgantas’ linų kintamumui. Nustatytas linų selekcijai naudingų mutantinių tipų skaičius. M_3 ir M_4 kartose nustatytas įvairių pakitimų paveldimumo laipsnis. Atrinkti linų mutantai, pasižymintys ūkiškai vertingomis savybėmis. Pateikiama kai kurių jų charakteristika.