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Variability of valuable economic traits in M_1 and M_2 sunflower generations influenced by dimethyl sulfate and γ-rays

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The development of effective mutation breeding methods and techniques for the modification of radiation and chemical injuries in plants is of vital importance. The effectiveness of induced mutagenesis is growing; therefore, our investigations were focused on the effects of mutagens on the growth and development of mutant generations and the variability of economic signs in M_1 and M_2 sunflower generations under the influence of dimethyl sulfate (DMS) and γ-rays. The aim of our research was to expand the genetic variability of important agronomic traits in M_1 and M_2 sunflower; identification of valuable mutants with altered characteristics in the next generations. Thus, in M_3 of lines Kh201V and Kh06-135V, we selected short mutants (122 cm vs 139 cm in the control) with lemon coloration of petals and multi-leafed mutants (201 leaves vs 23 in the control) with a modified shape and size of calathidium.

Keywords: variability, mutagenesis, mutant generation, sunflower, breeding

INTRODUCTION

Classical breeding methods based on the principles of selection and hybridization have achieved much success in the development of the theory of simulated evolution and enrichment of the plant gene pool. The application of modifiers with the known mechanisms of action on cellular structures and metabolic processes will help understand mechanisms of chromosome aberrations and solve the challenge of controlled mutagenesis, which is very important for creating starting material in genetics and breeding.

The aim of breeding is to achieve a genetic shift towards increased economic productivity of plants per crop area unit and product quality, i. e. to actively intervene in morphogenesis. The principal importance of experimental mutagenesis for breeding is determined by the ability to solve a number of objectives that cannot be achieved by other methods. This is, primarily, breeding for immunity, improvement of lines in terms of individual valuable economic traits, etc.

Despite the fact that scientists have proved mutagenic effects of radiation, chemicals, temperature, different internal and external factors (magnetic waves, ultraviolet, etc.) on living organisms, chemical supermutagens and ionizing radiation are the most effective.

Mutagens cause a wide range of heritable changes in sunflower; usually mutations impinge on morphology, oil quality, herbicide and low temperature resistance. I. A. Rapport (1981) concluded that using chemical mutagenesis, one could change the hereditary background of a plant, achieve a 'burst' of economically valuable traits in the short term.

The most important effect of chemical and physical mutagens is their potential to elicit a large number of recessive genes and cytoplasmic mutations, which affect sunflower variability. For sunflower, chemical mutagens are more effective than physical ones (Anaschenko, 1977).

A. A. Kalaydzhyan et al. (2007, 2009) studied effects of chemical mutagens on sunflower. They created a genetic collection consisting of 150 mutants, which differed by one or several traits. Basing on these mutants, they created 12 different models of sunflower. Treatment of seeds with 0.08% nitrosoethylurea generated mutants with a very short stem. After 18-hour treatment of sunflower seeds with 0.01% and 0.05% solutions, they obtained sunflower variants with overwintering rates of 63% (M-1248), 58% (M-1976) and 52% (M-2002). One of these mutants (M-1701) had a vegetation length of 45 days only. They also obtained compactoid mutants, mutants with high oil contents (56–57%), large seeds, short leafstalk, and white pollen.

From the breeding material obtained via chemical mutagenesis, the variety 'Pervenets' with a high oleic acid content was created (Soldatov, 1976).

Treatment of four accessions of the variety 'Peredovik' with ethyl methanesulfonate gave mutants with increased contents of palmitic acid in M_1 and M_2 generations (5–29%) (Velasco et al., 2008).

There was success in creating mutants resistant to some herbicides. Thus, using γ-radiation and chemical mutagens, mutants resistant to bifenox and glyphosate were derived (Berville et al., 1992); ethyl methanesulfonate mutagenesis produced mutants tolerant to imidazolinone (Sala et al., 2008).

Treating seeds of the variety 'Surya' with four doses of γ-rays (200 Gy) gave the greatest number of mutants: S. J. Jambhulkar and D. S. Joshua (1999) generated 27 morphological mutants in the M_2 generation. Among these mutations, there were novel features such as yellow leaf ribs, fasciations, wrinkled leaves, zigzag-shaped stems, zigzag-shaped semiflorets, stigma emergence and brown patch.

Treatment of isolated immature corcles with γ-rays (^{137}Cs) at the dose of 5 Gy derived mutants with a considerable variability of plant height, stem and calathidium diameters, bract length, oil content in seeds and 1000-seed weight (Encheva et al., 2002).

Irradiation of two sunflower genotypes with various doses of γ-rays led to a decrease in the seed germinability and survival percentage of mutant plants in the M_1 generation. In the M_2 generation, an increase in the mean values and variability of plant height, seed yield and oil content was observed (Jagadeesan et al., 2008).

A number of mutants in M_1, M_2, M_3, M_4 and M_5 generations resulted from the γ-irradiation of seeds of the variety 'VNIIMK 8931' (150 Gy; ^{60}Co). The mutant M95-674 is characterized by modified leaves and leafstalks (Christov, 1996).

Promising results (Škorić, 2015) were achieved when mutagenesis was used to improve the quality of oil, namely, a change in the ratio of fatty acids. Review of multiple literary sources showed that morphological traits were the most common object of mutations in sunflower.

The chlorotic apical part was produced through mutagenesis (Fambrini, Pugliesi, 1996). This was manifested as yellow cotyledons, the first pair of true green leaves, and later the apical part turned yellow.

As for resistance of sunflower to diseases, induced mutations have not brought the expected results yet. Positive results were obtained by M. F. De Oliveira et al. (2004) and J. Encheva et al. (2008) only for resistance to *Alternaria helianthi* and *Orobanhe*.

S. Cvejić et al. (2014, 2015) gamma-irradiated, fast neutron-irradiated or treated with ethyl methane sulfonate solution self-pollinated lines and obtained a lot of valuable mutants: an early-flowering form (L3ME), two short forms (L2MS and R1MS) and one tall form (R3MT), two forms with a high oil content (L1MO and R2MO) and one form with a branched stem (L4MBr).

P. Vijayata et al. (2016) studied the effects of chemical mutagens (0.05%, 0.10% and 0.15% EMS, and 0.01%, 0.02% and 0.03% SA) and gamma-rays (10 kR, 20 R and 30 kR) on the germination and field germinability of sunflower seeds of varieties 'Bhanu' and 'SS-56'. They concluded that germination in the both sunflower varieties was depressed due to the mutagenic treatment.

Chetankumar N. Banakar et al. (2015) investigated the effect of gamma-rays (10, 15 and 20 Kr)

on valuable economic characteristics of the parental lines of hybrids RSFH-130 (CMS-104B and R630), RFSH-1 (CMS-103B), and KBSH-44 (CMS-17B) in M_1 sunflower (*Helianthus annuus* L.). They found a correlation between the variability of traits and the mutagen dosing.

Analyzing published data on the results of mutagenesis in sunflower breeding, we found this trend promising, since mutants with altered morphological and agronomic traits had been obtained. Therefore, the aim of our research was to study the effects of mutagenic treatment on valuable traits in M_1 and M_2, to expand genetic diversity and to obtain a mutant of interest for breeding.

METHODS AND CONDITIONS

The field experiments were conducted in the experimental field of Kharkiv Dokuchaev National Agrarian University in 2013–2016.

To study the variability of homozygous lines in terms of a set of useful economic characteristics and the efficiency of mutagenesis, we investigated 12 self-pollinating sunflower lines bred at the Plant Production Institute nd. a. VYa Yuryev of NAAS of Ukraine.

Dry seeds of self-pollinating sunflower lines were treated with mutagens. In the studies on the effect of gamma irradiation on sunflower lines, dry seeds were single gamma-irradiated at the dose of 120 or 150 Gy in a cobalt chamber (irradiation source ^{60}Co). To study the effect of the chemical mutagen, we used the supermutagen dimethyl sulfate in concentrations of 0.01–0.05%. Sunflower seeds were treated with aqueous solutions of the chemical mutagen by the NN Zoz's method (1968). The exposure was 18 h. Dry untreated seeds were taken as the control.

Mutant plants selected for self-pollination were covered with parchment isolators one day prior to dehiscence of ray flowers. During the growing season, we determined the field germinability, carried out phenological observations, measured the plant height (20 days after anthesis) and the calathidium diameter, and counted the leaf number per plant.

To test null hypothesis, we used the Student's t-test. Calculations were performed using the Microsoft Office Excel 2010 and Statistica 7.

The starting material was prepared and studied as follows:

In 2014, 700 seeds were treated with mutagens in each variant. They were sown by 250 in each row. To avoid cross-pollination, plants were covered with parchment isolators.

In 2015, seeds harvested from self-pollinated plants were sown in single-line plots, each family separately. In each M_2 family, 3–5 plants were self-pollinated. By no means, all of changes in M_2 were hereditary, they could be modifications. To verify the nature of changes, forms selected in M_2 were sown by families in 2016. Further breeding can be conducted with M_3 mutants having valuable economic characteristics.

RESULTS AND DISCUSSION

When valuable economic features are studied in the mutant generation, an emphasis is put on the calathidium diameter, one of the most important parameters. Having examined the effects of two doses of γ-rays in M_1 and M_2 sunflower, we drew a conclusion on the positive impact of treatment on the calathidium size. Thus, the two-year data showed that the control calathidium diameter was 15.7 ± 3.1 cm (Lim = 10–23 cm). In 2014, the mean value of this parameter in the M_1 experimental plot with the accession Kh808 was 17.7 ± 3.7 cm (Lim = 12–24 cm) after 120 Gy irradiation, however, plants irradiated with 150 Gy responded negatively to the mutagen. The calathidium diameter decreased to 10.2 ± 2.1 cm (Lim = 8–14 cm). Having analyzed M_2 grown in 2015, we can consider the dynamics as positive, since after 120 Gy the calathidium diameter was 21.2 ± 2.9 cm (Lim = 16–24 cm), and after 150 Gy we observed a gain of 19.2 ± 5.4 cm (Lim = 8–28 cm) as compared with M_1 (Table 1).

In addition, we studied the effects of two concentrations of DMS on the test lines (Table 2). In general, there was no influence of DMS across 12 accessions; in some cases the calathidium diameter was smaller than the control one and one influenced by a physical mutagen. It is necessary to distinguish the mutant accession Mkh524V: its calathidium diameter was 16.6 ± 2.3 cm (Lim = 13–20 cm) and 16.4 ± 3.7 cm (Lim = 7–23 cm) in M_1 and M_2, respectively, for 0.01% solution, and 15.5 ± 2.2 cm (Lim = 12–18 cm) and 17.5 ± 4.3 cm

Table 1. **Effect of γ-rays on the calathidium diameter of M_1 and M_2 sunflower generations, cm, 2014–2015**

Doses of irradiation, Gy	Lines	Control	Lim	M_1	Lim	M_2	Lim
120	Od-973B	19.8 ± 3.8	14–28	19.7 ± 3.6	15–25	18.2 ± 3.6	10–28
	Kh808B	15.7 ± 3.1	10–23	17.7 ± 3.7	12–24	21.2 ± 2.9	16–24
	Kh1002B	17.6 ± 2.3	12–23	17.6 ± 5.1	11–24	22.5 ± 5.6	10–35
	Kh1008B	18.7 ± 2.6	13–27	15.4 ± 3.6	10–21	17.9 ± 5.0	5–31
	Mkh-845B	15.1 ± 2.5	11–22	16.0 ± 2.3	13–20	18.3 ± 5.0	8–33
	Kh-08-16V	12.0 ± 1.3	9–14	9.7 ± 2.2	6–13	12.1 ± 2.8	7–20
	Kh-134V	11.1 ± –1.2	9–13	11.5 ± 5.8	7–23	12.8 ± 2.2	8–23
	Kh-785V	10.5 ± 1.3	7–13	10.6 ±1.8	8–14	11.4 ± 1.5	7–15
	Kh IR1G (Kh 201V)	11.1 ± 1.0	10–14	11.2 ± 4.1	6–18	11.6 ± 2.0	7–17
150	Od-973B	19.8 ± 3.8	14–28	13.3 ± 1.5	11–15	14.7 ± 2.6	10–19
	Kh808B	15.7 ± 3.1	10–23	10.2 ± 2.1	8–14	19.2 ± 5.4	8–28
	Kh1002B	17.6 ± 2.3	12–23	15.2 ± 4.0	8–20	20.8 ± 5.7	10–40
	Kh1008B	18.7 ± 2.6	13–27	10.9 ± 3.0	7–17	19.2 ± 5.6	7–32
	Mkh-845B	15.1 ± 2.5	11–22	16.7 ± 1.3	15–19	19.5 ± 5.5	8–29
	Kh-08-16V	12.0 ± 1.3	9–14	16.7 ± 1.2	15–19	13.2 ± 2.2	8–18
	Kh-134V	11.1 ± –1.2	9–13	11.7 ± 2.1	9–15	13.7 ± 1.8	10–17
	Kh06-135V	17.7 ± 3.2	12–29	12.7 ± 2.4	9–15	21.5 ± 7.2	8–36
	Kh-785V	10.5 ± 1.3	7–13	14.4 ± 3	11–20	10.5 ± 1.7	8–15
	KhIR1G (Kh201V)	11.1 ± 1.0	10–14	12.3 ± 1.3	10–15	11.4 ± 2.1	7–15

Table 2. **Effect of chemical mutagen dimethyl sulfate on the calathidium diameter of M_1 and M_2 sunflower generations, cm, 2014–2015**

Concentration, %	Lines	Control	Lim	M_1	Lim	M_2	Lim
0.01	Od-973B	19.8 ± 3.8	14–28	17.7 ± 1.5	16–20	18.5 ± 4.2	9–27
	Kh808B	15.7 ± 3.1	10–23	16.1 ± 1.8	14–20	14.4 ± 2.8	6–22
	Kh1002B	17.6 ± 2.3	12–23	17.1 ± 0.9	16–18	15.7 ± 4.0	7–30
	Kh1008B	18.7 ± 2.6	13–27	20.4 ± 0.8	19–22	14.1 ± 3.2	7–25
	Mkh-524B	14.5 ± 2.8	10–23	16.6 ± 2.3	13–20	16.4 ± 3.4	7–23
	Mkh-845B	15.1 ± 2.5	11–22	15.5 ± 2.0	13–19	14.9 ± 3.6	8–23
	Kh-08-16V	12.0 ± 1.3	9–14	13.7 ± 0.8	13–15	11.8 ± 1.0	10–14
	Kh-134V	11.1 ± –1.2	9–13	11.9 ± 1.3	10–14	11.3 ± 0.9	9–14
	Kh06-135V	17.7 ± 3.2	12–29	19.6 ± 2.0	15–21	17.2 ± 3.0	10–22
	Kh-785V	10.5 ± 1.3	7–13	11.3 ± 1.3	10–13	10.8 ± 1.0	7–12
	Kh-1334V	15.5 ± 1.3	13–18	15.4 ± 1.1	14–17	15.1 ± 2.3	11–20
	Kh IR1G (Kh 201V)	11.1 ± 1.0	10–14	11.0 ± 0.9	10–12	11.6 ± 1.6	7–15
0.05	Od-973B	19.8 ± 3.8	14–28	17.1 ± 2.0	14–22	20.0 ± 4.5	11–30
	Kh808B	15.7 ± 3.1	10–23	16.3 ± 1.1	15–18	13.5 ± 3.1	6–20
	Kh1002B	17.6 ± 2.3	12–23	14.6 ± 2.8	11–20	14.2 ± 2.4	8–21
	Kh1008B	18.7 ± 2.6	13–27	20.8 ± 1.4	19–23	15.1 ± 3.1	9–24
	Mkh-524B	14.5 ± 2.8	10–23	15.5 ± 2.2	12–18	17.5 ± 4.3	5–30
	Mkh-845B	15.1 ± 2.5	11–22	16.9 ± 2.4	13–20	19.1 ± 3.8	10–28
	Kh-08-16V	12.0 ± 1.3	9–14	12.6 ± 1.5	10–15	11.6 ± 1.2	7–15
	Kh-134V	11.1 ± –1.2	9–13	11.0 ± 1.2	10–13	10.7 ± 1.0	8–14
	Kh06-135V	17.7 ± 3.2	12–29	19.5 ± 1.8	16–21	15.8 ± 3.0	10–23
	Kh-785V	10.5 ± 1.3	7–13	11.1 ± 1.1	10–13	12.1 ± 1.4	7–15
	Kh-1334V	15.5 ± 1.3	13–18	17.3 ± 3.1	12–24	15.1 ± 1.8	12–20
	Kh IR1G (Kh 201V)	11.1 ± 1.0	10–14	10.7 ± 0.9	10–12	11.1 ± 1.9	5–18

(Lim = 5–30 cm) in M_1 and M_2, respectively, for 0.05% solution, thus exceeding the control value 14.5 ± 2.8 cm (Lim = 10–23 cm).

The second significant valuable economic feature is the plant height, which is important for mechanized harvest. In general, the test accessions responded to the physical mutagen by a decrease in the plant height, which is a positive phenomenon.

For example, the tall accession Kh1002V with the mean height of 166.1 ± 8.6 cm (Lim = 146–185 cm) had 146.2 ± 9.7 cm (Lim = 135–163 cm) in 120 Gy M_1, 136.9 ± 9.9 cm (Lim = 126–160 cm) in 150 Gy M_1, and 155.4 ± 18.8 cm (Lim = 110–186 cm) in 120 Gy M_2. At the same time, after 150 Gy irradiation the plant height was 173.1 ± 16.0 cm (Lim = 120–200 cm), exceeding the control value (166.1 ± 8.6 cm, Lim = 146–185 cm).

The test accession KhH808V had the height of 142.9 ± 10.7 cm (Lim = 110–160 cm); as a result of γ-irradiation, M_1 plants were significantly lower than the control: 120 Gy, 104.3 ± 11.8 cm (Lim = 93–130 cm), 150 Gy, 130.0 ± 10.9 cm (Lim = 103–144 cm); in M_2, the plant height was 123.2 ± 9.1 cm (Lim = 110–140 cm) and 130.8 ± 20.5 cm (Lim = 65–168 cm) for 120 Gy and 150 Gy, respectively (Table 3).

As to the influence of DMS on Kh808V, the height was depressed in 0.01% M_1 (136.3 ± 3.3 cm (Lim = 130–140 cm)) and augmented in 0.05% M_1 (146.9 ± 5.0 cm (Lim = 140–155 cm)) as compared to the control – 142.9 ± 10.7 cm (Lim = 110–160 cm). In M_2, we recorded a slight reduction in the height for the both concentrations: 0.01% – 141.6 ± 15.5 cm (Lim = 64–172 cm), and 0.05% – 137.8 ± 12.6 cm (Lim = 90–160 cm).

The mutant line Mkx524V is noteworthy, since it responded to DMS and γ-rays by decreasing its height: in 0.01% M_1 it was 121.5 ± 15.5 cm (Lim = 104–160 cm); in 0.05% M_1 it was 113.4 ± 10.5 cm (Lim = 100–134 cm) related to the control height of 135.9 ± 8.8 cm (Lim = 112–150 cm). This change was also noted in M_2, hence, it can be called a mutation: in 0.01% M_2 the height was 108.5 ± 14.5 cm (Lim = 60–136 cm); in 0.05% M_2 it was 115.0 ± 13.1 cm (Lim = 75–140 cm). Accessions Kh1002V, KhH1334V, Kh758V, and Kh1008V were exceptions, because they were slightly taller than the control plants.

Thus, we may conclude that the effects of chemical mutagen and γ-rays on the sunflower height in M_1 and M_2 were significant, since all the 12 test accessions responded to mutagens by decreasing their height (Table 4).

Table 3. **Effect of γ-rays on the plant height of M_1 and M_2 sunflower generations, cm, 2014–2015**

Doses of irradiation, Gy	Lines	Control	Lim	M_1	Lim	M_2	Lim
120	Od-973B	155.0 ± 8.2	140–165	128.3 ± 8.6	119–141	138.2 ± 9.0	115–158
	Kh808B	142.9 ± 10.7	110–160	104.3 ± 11.8	93–130	123.9 ± 9.1	110–140
	Kh1002B	166.1 ± 8.6	146–185	146.2 ± 9.7	135–163	155.4 ± 18.8	110–186
	Kh1008B	143.0 ± 14.2	120–162	139.5 ± 6.6	128–150	150.5 ± 17.1	65–195
	Mkh-845B	126.9 ± 6.4	110–145	106.1 ± 3.0	100–110	123.8 ± 12.8	82–150
	Kh-08-16V	142.9 ± 6.9	127–158	102.6 ± 4.3	96–110	152.3 ± 13.3	110–177
	Kh-134V	152.2 ± 3.0	145–157	112.2 ± 4.6	90–159	133.5 ± 11.3	97–150
	Kh-785V	127.5 ± 6.1	110–144	106.6 ± 9.2	93–118	131.4 ± 9.8	100–151
	Kh IR1G (Kh 201V)	169.7 ± 6.3	155–185	115.6 ± 14.7	98–140	158.4 ± 14.6	110–180
150	Od-973B	155.0 ± 8.2	140–165	122.6 ± 7.5	115–135	143.2 ± 4.9	134–152
	Kh808B	142.9 ± 10.7	110–160	130.0 ± 10.9	103–144	130.8 ± 20.5	65–168
	Kh1002B	166.1 ± 8.6	146–185	136.9 ± 9.9	127–160	173.1 ± 16.0	120–200
	Kh1008B	143.0 ± 14.2	120–162	111.0 ± 7.8	96–121	147.6 ± 21.6	75–175
	Mkh-845B	126.9 ± 6.4	110–145	106.4 ± 5.1	100–115	119.9 ± 15.3	60–155
	Kh-08-16V	142.9 ± 6.9	127–158	102.3 ± 3.9	95–108	139.7 ± 12.0	90–160
	Kh-134V	152.2 ± 3.0	145–157	119.9 ± 4.7	112–130	138.0 ± 11.3	110–157
	Kh06-135V	153.9 ± 8.9	135–170	131.7 ± 10.0	110–143	144.5 ± 19.1	100–175
	Kh-785V	127.5 ± 6.1	110–144	145.2 ± 29.0	115–180	128.8 ± 10.9	90–144
	Kh IR1G (Kh 201V)	169.7 ± 6.3	155–185	102.0 ± 3.8	95–107	150.8 ± 13.6	105–178

Table 4. **Effect of chemical mutagen dimethyl sulfate on the plant height of M_1 and M_2 sunflower generations, cm, 2014–2015**

Concentration, %	Lines	Control	Lim	M_1	Lim	M_2	Lim
0.01	Od-973B	155.0 ± 8.2	140–165	150.4 ± 5.8	140–160	147.5 ± 10.3	110–167
	Kh808B	142.9 ± 10.7	110–160	136.3 ± 3.3	130–140	141.6 ± 15.5	64–172
	Kh1002B	166.1 ± 8.6	146–185	150.2 ± 5.2	140–158	172.1 ± 10.0	143–188
	Kh1008B	143.0 ± 14.2	120–162	149.6 ± 7.3	140–158	142.6 ± 18.7	86–165
	Mkh-524B	135.9 ± 8.8	112–150	121.5 ± 15.5	104–160	108.5 ± 14.5	60–136
	Mkh-845B	126.9 ± 6.4	110–145	114.7 ± 10.2	100–134	109.1 ± 13.7	65–136
	Kh-08-16V	142.9 ± 6.9	127–158	128.2 ± 8.5	110–136	129.9 ± 11.7	90–155
	Kh-134V	152.2 ± 3.0	145–157	156.3 ± 8.4	144–175	143.1 ± 9.5	115–165
	Kh06-135V	153.9 ± 8.9	135–170	147.2 ± 5.0	140–153	138.9 ± 12.2	104–160
	Kh-785V	127.5 ± 6.1	110–144	131.5 ± 8.8	116–142	127.5 ± 9.3	90–150
	Kh-1334V	160.1 ± 6.9	147–170	160.4 ± 3.1	153–165	162.7 ± 8.9	137–180
	KhIR1G (Kh 201V)	169.7 ± 6.3	155–185	173.2 ± 2.6	170–177	157.2 ± 11.6	130–175
0.05	Od-973B	155.0 ± 8.2	140–165	145.1 ± 10.6	125–158	147.1 ± 14.2	105–174
	Kh808B	142.9 ± 10.7	110–160	146.9 ± 5.0	140–155	137.8 ± 12.6	90–160
	Kh1002B	166.1 ± 8.6	146–185	157.8 ± 5.2	150–168	168.6 ± 8.9	140–185
	Kh1008B	143.0 ± 14.2	120–162	135.1 ± 13.9	100–150	147.7 ± 12.5	110–175
	Mkh-524B	135.9 ± 8.8	112–150	113.4 ± 10.5	100–134	115.0 ± 13.1	75–140
	Mkh-845B	126.9 ± 6.4	110–145	113.1 ± 4.1	110–120	119.0 ± 10.1	90–137
	Kh-08-16V	142.9 ± 6.9	127–158	134.9 ± 11.0	120–161	132.7 ± 10.5	95–155
	Kh-134V	152.2 ± 3.0	145–157	151.7 ± 8.1	140–170	143.1 ± 8.4	115–157
	Kh06-135V	153.9 ± 8.9	135–170	139.4 ± 6.0	132–153	146.7 ± 14.4	105–173
	Kh-785V	127.5 ± 6.1	110–144	124.9 ± 9.8	110–140	136.4 ± 9.9	115–160
	Kh-1334V	160.1 ± 6.9	147–170	162.9 ± 3.1	157–166	163.5 ± 6.3	140–180
	KhIR1G (Kh201V)	169.7 ± 6.3	155–185	162.3 ± 7.1	148–172	159.0 ± 8.0	140–178

Valuable developmental abnormalities were distinguished throughout the growing season in the experimental mutagenesis plots. Some of them, such as the lemon apical point in the line Kh134V (0.05% DMS), red tint of leaves in the line Kh134V (0.01% DMS), lemon semiflorets in the line KhIR1G (Kh201V) (γ-rays 120 Gy) were inherited from M_1 to M_2. Chlorophyll plants with deformed or double leaves, leaves without the midrib, multiple calathidiums, plants with two apical points were frequent. The calathidium shape in the line Kh1008V was modified (M_2: γ-rays, 120 Gy) (Table 5).

DMS and γ-rays suppressed a valuable for plants trait, the leaf number, indicating their negative impact on the test accessions, because the leaf number plays an important role in photosynthesis.

The test accession KhIR1G (Kh201V) is noteworthy because it had the increased number of leaves, a valuable for breeders phenomenon, in M_2 after the 0.05% DMS treatment. We investigated and selected accessions with 85–91 leaves. For comparison, the control had 24 ± 4 leaves (Lim = 18–33 leaves) (Table 6).

Mutants with altered characteristics were distinguished in M_3. Thus, we distinguished short forms with a height of 122 ± 9.6 cm (control was 139.8 ± 5.5 cm tall) with lemon petals (control petals were orange) from the gamma-irradiated at 150 Gy line Kh201. The aftereffect of 0.05% DMS in the same line resulted in tall (151.4 ± 7.1 cm while the control height was 139.8 ± 5.5 cm) multi-leafed mutants (201 ± 32 leaves vs 23 ± 3 leaves in the control) with a modified shape and size of calathidium (21 ± 1.5 cm vs 13 ± 1.3 cm in the control) (Table 7).

Table 5. **Effect of γ-rays on the leaf number of M_1 and M_2 sunflower generations, cm, 2014–2015**

Doses of irradiation, Gy	Lines	Control	Lim	M_1	Lim	M_2	Lim
120	Od-973B	29 ± 2.3	24–34	20 ± 2.7	16–24	23 ± 4	14–33
	Kh808B	26 ± 2.7	21–34	22 ± 1.9	19–24	20 ± 3	17–23
	Kh1002B	27 ± 2.8	20–38	22 ± 2.9	18–27	21 ± 4	11–27
	Kh1008B	23 ± 1.9	20–26	20 ± 2.3	17–25	23 ± 3	14–31
	Mkh-845B	25 ± 2.9	17–29	20 ± 1.2	18–22	27 ± 3	17–35
	Kh-08-16V	24 ± 2.4	18–30	12 ± 1.8	10–15	25 ± 4	16–33
	Kh-134V	26 ± 3.0	20–34	19 ± 5.0	12–25	22 ± 3	15–31
	Kh-785V	27 ± 3.0	20–32	18 ± 3.2	13–22	22 ± 3	17–29
	KhIR1G (Kh201V)	24 ± 4.1	18–33	26 ± 5.8	13–34	18 ± 2	13–26
150	Od-973B	29 ± 2.3	24–34	18 ± 1.1	16–20	24 ± 4	18–33
	Kh808B	26 ± 2.7	21–34	18 ± 2.0	14–21	20 ± 3	10–29
	Kh1002B	27 ± 2.8	20–38	18 ± 2.2	15–22	24 ± 3	13–33
	Kh1008B	23 ± 1.9	20–26	15 ± 2.8	10–18	23 ± 3	16–29
	Mkh-845B	25 ± 2.9	17–29	17 ± 2.1	13–20	27 ± 5	11–38
	Kh-08-16V	24 ± 2.4	18–30	19 ± 1.4	17–21	23 ± 3	16–28
	Kh-134V	26 ± 3.0	20–34	16 ± 3.6	11–21	24 ± 3	16–28
	Kh06-135V	27 ± 3.5	16–32	18 ± 1.3	16–19	21 ± 4	10–27
	Kh-785V	27 ± 3.0	20–32	18 ± 0.9	16–19	23 ± 3	16–29
	KhIR1G (Kh201V)	24 ± 4.1	18–33	18 ± 1.7	16–21	17 ± 3	12–29

Table 6. **Effect of chemical mutagen dimethyl sulfate on the leaf number of M_1 and M_2 sunflower generations, cm, 2014–2015**

Concentration, %	Lines	Control	Lim	M_1	Lim	M_2	Lim
0.01	Od-973B	29 ± 2.3	24–34	29 ± 2.3	26–32	26 ± 3	15–33
	Kh808B	26 ± 2.7	21–34	25 ± –1.5	22–26	25 ± 4	14–33
	Kh1002B	27 ± 2.8	20–38	25 ± 1.1	24–27	24 ± 2	16–29
	Kh1008B	23 ± 1.9	20–26	27 ± 1.2	26–29	21 ± 3	15–27
	Mkh-524B	27 ± 1.9	21–30	27 ± 3.7	22–30	24 ± 3	16–30
	Mkh-845B	25 ± 2.9	17–29	28 ± 2.1	24–30	21 ± 4	13–31
	Kh-08-16V	24 ± 2.4	18–30	27 ± 1.9	24–30	25 ± 3	13–33
	Kh-134V	26 ± 3.0	20–34	26 ± 1.6	23–28	26 ± 3	17–32
	Kh06-135V	27 ± 3.5	16–32	28 ± 3.0	22–33	28 ± 4	16–37
	Kh-785V	27 ± 3.0	20–32	30 ± 1.6	27–32	24 ± 2	17–27
	X-1334B	28 ± 2.3	22–35	29 ± 2.1	26–32	27 ± 2	21–32
	KhIR1G (Kh201V)	24 ± 4.1	18–33	28 ± 1.8	26–30	21 ± 4	12–31
0.05	Od-973B	29 ± 2.3	24–34	28 ± 2.1	26–32	26 ± 3	19–34
	Kh808B	26 ± 2.7	21–34	28 ± 2.0	25–30	20 ± 3	10–28
	Kh1002B	27 ± 2.8	20–38	28 ± 2.5	24–32	23 ± 3	15–33
	Kh1008B	23 ± 1.9	20–26	25 ± 3.6	19–30	21 ± 3	13–28
	Mkh-524B	27 ± 1.9	21–30	29 ± 2.1	26–32	21 ± 3	12–27
	Mkh-845B	25 ± 2.9	17–29	29 ± 2.5	24–32	20 ± 3	13–29
	Kh-08-16V	24 ± 2.4	18–30	25 ± 1.5	22–26	26 ± 3	16–32
	Kh-134V	26 ± 3.0	20–34	29 ± 1.6	27–32	25 ± 3	18–32
	Kh06-135V	27 ± 3.5	16–32	28 ± 3.4	23–34	29 ± 4	18–37
	Kh-785V	27 ± 3.0	20–32	24 ± 2.8	20–28	22 ± 4	13–27
	Kh-1334V	28 ± 2.3	22–35	31 ± 2.1	28–34	28 ± 3	21–36
	KhIR1G (Kh201V)	24 ± 4.1	18–33	21 ± 2.7	18–26	25 ± 12	9–91

Table 7. **Mutant forms in M_3 sunflower with altered plant height, cm, 2016**

Family	Plant height, h, cm	Lim	Variation coefficient, V, %	Student's-test, t
Kh06-135V				
Control	157.9 ± 4.4	151–165	2.8	–
(DMS 0.01%) multi-leafed	93.2 ± 9.5*	86–110	10.2	18.3 > 2.14
(DMS 0.01%) 96/97	88.2 ± 14.5*	71–100	16.4	14.4 > 2.16
(DMS 0.01%) 63 Lemon	65.6 ± 12.9*	45–79	19.6	21.2 > 2.13
Kh201 V				
Control	139.8 ± 5.5	133–150	3.9	–
(DMS 0.05%) 742b	151.4 ± 7.1*	140–164	4.7	4.1 > 2.1
(DMS 0.05%) 727	109.1 ± 7.3*	100–120	6.7	10.5 > 2.11
(γ-rays 120 Gy) 1133 Lemon	122 ± 9.6*	115–136	7.8	4.7 > 2.16
(γ-rays 150 Gy) 1159	116.8 ± 6.1*	110–125	5.2	7.4 > 2.16
(γ-rays 150 Gy) 1143	108.3 ± 4.9*	100–115	4.5	12.8 > 2.12

Footnote. * significant differences, $t_{fact.} > t_{teor.}$

Mutagens affected the diameter of calathidium in M_3, which had an impact on its size. Often, depending on changes in plant height, the calathidium diameter either decreased or remained the same as the control one. The multi-leafed mutant 742 selected from the line Kh201V (0.05% DMS), in which the calathidium diameter and shape were substantially different from the control (21.1 ± 1.5 cm and 12.9 ± 1.3 cm, respectively), was an exception. Mutants 96/97 (0.01% DMS) and 63 Lemon (0.01% DMS) with a modified habit, height and petal color had significantly smaller calathidiums (8.6 ± 2.1 cm and 6.7 ± 0.5 cm, respectively) as compared to the control 15.9 ± 2.1 cm (Table 8).

CONCLUSIONS

The study of M_1 and M_2 sunflower found that γ-irradiation influenced the calathidium diameter and plant height of the test lines, either reducing or increasing these parameters. No consistent pattern in the calathidium diameter was observed with the DMS aftereffect, and the plant height slightly decreased in this case. In M_2 of some lines, changes of other traits, such as lemon coloration of apical point and ray flowers, red shade of leaves multiple calathidiums, were noticed. It was found that mutagens induced reduction in the leaf number, which is undesirable, as it reduces the rate of photosynthesis. At the same

Table 8. **Mutant forms in M_3 sunflower with altered calathidium diameter, cm, 2016**

Family	Calathidium diameter, d, cm	Lim	Variation coefficient, V, %	Student's-test, t
Kh201V				
Control	**12.9 ± 1.3**	**11–15**	**9.7**	–
742 multi–leafed (DMS 0.05%)	21.1 ± 1.5*	20–24	7.1	12.6 > 2.1
727 (DMS 0.01%)	12.0 ± 1.1*	10–13	9.6	2.7 ≥ 2.11
1133 Lemon (γ-rays 120 Gy)	11.6 ± 2.2*	10–14	1.9	2.0 > 2.1
1143 (γ-rays 150 Gy)	11.9 ± 1.8*	10–14	15.2	1.7 > 2.12
1159 (γ-rays 150 Gy)	12.6 ± 1.3*	12–15	10.6	0.7 > 2.16
Kh06-135V				
Control	**15.9 ± 2.1**	**13–20**	**13.1**	–
96/97 (DMS 0.01%)	8.6 ± 2.1*	7–12	24.1	6.4 > 2.16
63 Lemon (DMS 0.01%)	6.7 ± 0.5*	6–7	7.8	11.9 > 2.13
6 (DMS 0.01%)	12.6 ± 0.9*	12–14	7.1	3.3 > 2.16

time, we distinguished the multi-leafed accession KhIR1H (Kh201V) with 85–91 leaves in comparison with 24 leaves in the control in M_2.

In M_3, we distinguished mutants with altered hereditary traits. It should be noted that the lines Kh201V and Kh06-135V turned out to be the most promising, judging by the mutant output.

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SAULĖGRĄŽOS EKONOMIŠKAI SVARBIŲ POŽYMIŲ KINTAMUMAS M_1 IR M_2 KARTOSE PAVEIKUS DIMETILSULFATU IR γ-RADIACIJA

S a n t r a u k a

Augalų selekcijoje svarbu parengti efektyvius mutantų kūrimo cheminės ir radiacinės mutagenezės būdu metodus bei mutantų atrankos technologijas. Tyrimas orientuotas į mutagenų γ-radiacijos ir dimetilsulfato (DMS) efektyvumo palyginimą vertinant M_1 ir M_2 mutantų kartų augimą, vystymąsi ir jų ekonomiškai svarbių požymių kitimą. Tyrimo tikslas – išplėsti ekonomiškai svarbių požymių įvairovę M_1 ir M_2 kartose; identifikuoti vėlesnėse kartose vertingus mutantus su pakeistomis savybėmis. M_3 kartoje iš linijų Kh201V ir Kh06-135V buvo atrinkti trumpastiebiai mutantai (122 cm; kontrolė – 139 cm); mutantai su citrinos spalvos vainiklapiais; gausialapiai mutantai (201 lapai; kontrolė – 23 lapai); mutantai su pakitusia graižo forma ir dydžiu.

Raktažodžiai: kintamumas, mutagenezė, mutantų kartos, saulėgrąža, selekcija