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Generation, Evaluation, and Prospects of Further Use of Mutations Based on New Homozygous Self-Pollinated Sunflower Lines

Victoria Mykhailenko, Viktor Kyrychenko, Alexander Bragin and Dmitry Chuiko

Abstract

A majority of sunflower lines and hybrids were based on starting material obtained by traditional methods; so the issues of developing new trends in extending the genetic diversity of this crop require constant attention of scientists. At present, induced mutagenesis along with hybridization has become a leading method for generating new forms of crops. Their success depends largely on availability and assortment of starting material. Induction of mutations is a way to create it. The main value of induced mutagenesis for breeding is determined by opportunities to solve problems that are impossible or difficult to solve by traditional methods. The choice of an effective concentration (dose) of a mutagen is very important, since the frequency and range of mutations depend not only on the mutagen itself but also on its dose and exposure. In addition, it is relevant to search for new mutagens with reduced harmful effects at the same level of mutability. Cytological analysis of chromosomal aberrations is an important method of evaluation and identification of mutagenic effects. In this section, studies into chemical and physical mutagenesis in breeding, exemplified by new modern homozygous self-pollinated sunflower lines, are summarized; methodical recommendations on the use of induced mutagenesis in sunflower breeding are presented; and methods of generation, investigation, and further use of mutations are rationalized.

Keywords: gamma rays, dimethyl sulfate, mutagenesis, meiosis, mutation, self-pollinated line, breeding, sunflower

1. Introduction

In comparison with other oil crops, sunflower produces the highest oil yield per unit area (on average 750 kg/ha in Ukraine), which makes this crop a major oil crop. The oil content in seeds of released hybrids is 50–52%, and in breeding hybrids—up to 60%.

The nutritional value of sunflower oil is determined by high content of polyunsaturated fatty linoleic acid (55–60%), which has a significant biological

activity and accelerates the metabolism of cholesterol esters in the body, which has a positive effect on health. Sunflower oil also contains ingredients that are very valuable to the human body, such as phosphatides, sterols, and vitamins (A, D, E, K). The nutritional value of sunflower seeds per 100 g is as follows: energy, 2445 kJ; proteins, 20.8 g; fats, 51.5 g (of which saturated fats account for 4.5 g; monounsaturated ones, 18.5 g; and polyunsaturated ones, 23.1 g); and carbohydrates, 20 g. This makes sunflower a valuable food product.

Induced mutagenesis allows developing new starting material with various morphological and physiological features and biochemical parameters, increasing the frequency and expanding the assortment of original mutations within a short time.

Mutations are a source of expansion of the genetic diversity of sunflower, which in its turn is a starting material for the breeding of this crop. Radiation and chemical mutagens are used to produce artificial mutations in sunflower [1].

The strongest chemical mutagens (supermutagens), which cause a several hundred-fold increase in the frequency of mutations, include ethyleneamine, diethyl sulfate, dimethyl sulfate, nitrosoethylurea, nitrosomethylurea, hydrogen peroxide, etc. [2].

Since Wetterer's first attempts to gamma-irradiate sunflower seeds in 1911, Shull and Mitchell's experiments in 1933 [3], Soldatov's achievements [4], reports of contemporary scientists Kalaydzhan [5], Lacombe [6], Soroka [7], Cvejic [8, 9], Lyakh [10], Vasin [11, 12], Kyrychenko [13], Škorić [14], and many others, a considerable progress have been achieved in enriching the sunflower gene pool by induced mutagenesis. However, despite considerable advances, due to continuous refreshment of starting material, induced mutagenesis has been and remains an important method for developing new and improving existent starting material in breeding.

Our purpose was to obtain self-pollinated sunflower lines with genetic mutations induced by chemical and physical mutagens that can be used to improve features of the sunflower crop and to develop methodological approaches for studying mutant generations.

2. Means and mechanisms of experimental mutation induction

When researchers obtain and control new hereditary changes in plants during their experiments, some completely new possibilities to create breeding initial material appear.

Since Watson and Crick decoded the structure of DNA, characterized the mechanism of its replication and discovered the system of recording genetic information, highlighting the genetic nature of mutations, it became evident that the primary cause of any mutation is the primary disorders in the DNA structure, which are in the process of cell metabolism can be realized in true mutations or repaired and restored to their original state.

Primary abnormalities induced in hereditary structures of an organism under the influence of natural or artificial factors can cause the appearance of two types of mutations—point ones, caused by disorders in the original structures of the DNA molecule, and chromosomal ones, caused by qualitative or quantitative changes in the chromosomal systems of cells.

Primary disorders in DNA structures are not repaired to their original state; they initiate the processes of gene (point) mutation formation. Such disorders include replacement of nitrogen base pairs (transversions); the inclusion of additional complementary pairs of nucleotides (duplication); loss of nucleotide pairs in the structure of a DNA molecule (deletions); 180° rotation of nucleotide pairs (inversions), etc.

Initial DNA integrity disorders may result in chromosome rupture. In this case open sections of chromosomal filaments can be combined reaching their original state or form new combinations. Thus, there are chromosomal mutations that are characterized by a wide diversity.

Mutations can attach a molecule of sugar (deoxyribose), phosphate, or a nitrogenous base to the nucleotides. For example, despite the fact that deoxyribose is the only sugar group in DNA, it is not desirable to exclude the possibility of accidental incorporation of individual ribose molecules into DNA. In such cases, the phosphate and nitrogenous bases of the ribonucleotide may be like those of the deoxyribonucleotides. Phosphoric acid, as a component of DNA and RNA, may contain radioactive P32 atom instead of a normal phosphorus one.

Disorders induced by irradiation in DNA molecules can affect phosphodiester, sugar-phosphate, glycosyl, and other chemical bonds. As a result, single or double-thread breaks occur, as well as the destruction of nitrogenous bases. For example, when dry seeds are irradiated, the nitrogenous bases included in the DNA structure may be converted into thymine, guanine radicals, etc., which are sufficiently stable in the dry state and sufficiently reactive when wetted.

The alkylating compounds including DMS are a source to introduce the methyl radicals (CH₃), ethyl (C₂H₅), etc. into the molecules, thereby providing an alkylation reaction. They are characterized by a wide range of mutagenic effects, inducing simple and complex substitutions as well as breaks in DNA molecules. All nitrogenous bases, phosphoric acid residue, and even deoxyribose residue are alkylated. As a result of alkylation reactions, the purine bases are most likely to fall out DNA strand, causing the formation of voids at the corresponding points in the molecule. It is obvious that the mechanism of mutagenesis in the alkylation of DNA bases is associated with a violation in the accuracy of the auto-reproduction of DNA molecules.

All these events eventually result in changes within DNA molecules that manifest themselves as mutations, most of which are lethal. However, many mutations are viable. They are involved in the process of gene recombination, and as a result they are integrated in their functions with other genes of the genotype where they originated.

3. Research methodology: selection of starting material

The plant genotype has a significant effect on the specificity and level of mutations. Therefore, selection of starting material plays a significant role in obtaining valuable mutants. Generally, the best area-specific forms are recommended to use as sources, which need refining in terms of individual characteristics and features. Constant self-pollinated forms are the best for mutational breeding, as their mutations can be easily and reliably identified. Therefore, working with mutagenesis, one should apply different methods of isolation of nurseries and mutant plants in order to prevent biological contamination and occurrence, along with mutations, of possible recombinations.

Twelve new homozygous, self-pollinated sunflower lines from a genetic collection of the Plant Production Institute named after VYa. Yuriev, which are of breeding value and differ in several morphological and biochemical features, were taken as study objects.

Chemical supermutagen dimethyl sulfate (DMS) and gamma rays were used to induce genetic variability. Two hundred fifty seeds were used in each variant of treatment.

As to chemical mutagenesis, seeds in capron partly loosened sacs were soaked in 0.01 and 0.05% DMS solutions (prepared on distilled water, as some mutagens tend to degrade rapidly in tap water). We prepared these solutions under a hood, wearing rubber gloves: crushed an ampoule with mutagen in water. Depending on the object, the treatment time ranges 2–24 h. With sunflower seeds, the exposure was 18 hours, with periodic stirring. The mutagen/treated seeds ratio (volume/weight) was 10:1. In addition, to accelerate the process of solution penetration through the seed coat, it is recommended to ultrasound seeds for a short time (1–7 min) [15]. After treatment of seeds, in order to reduce the damaging effect, we washed them out for 1 h in running tap water and then sowed in soil on the same day.

Dimethyl sulfate (DMS) is a chemical supermutagen, an alkylating compound, which breaks chromosomes, leading to a large number of chromosomal inversions.

Studying physical mutagenesis on new sunflower lines, we used gamma rays from the radioactive isotope Co60, which has a relatively high irradiation uniformity. Dry seeds were once irradiated on a “Theratron Elit 80” Ionizing Radiation Source Cobalt 60 at Kharkiv Regional Oncology Hospital.

In a research on induced mutagenesis, a great attention is paid to concentrations (doses) of mutagens, which affect the number and quality of mutations; therefore, we used the most effective for agricultural crops DMS concentrations (0.01 and 0.05%) and doses of gamma rays (120 and 150 Gy).

Seeds of corresponding sunflower lines soaked in distilled water were used as controls.

When working with mutagens, which are poisonous and sometimes volatile, one should strictly follow the safety regulations and have appropriate equipment and rooms [16, 17].

4. Generation, evaluation, and further use of mutations: M₁ generation

Mutagen-treated seeds were sown in mutant nurseries: M₁ nursery (area = 20 m²; single-row plots comprising 250 plants each), M₂ nursery (area = 40 m²; single-row plots comprising 25 plants each), and M₃ nursery (area = 50 m²; single-row plots comprising 25 plants each). The sowing scheme was 70 × 25 cm. Seeds were sown with manual planters within the optimal timeframe (2nd–3rd 10 days of May). Winter wheat was the forecrop. Mutant plants in the experimental plots were harvested by cutting and manually threshed.

We observed the expected decrease in the field germinability in the M₁ generation, and the higher concentration or dose of the mutagenic factor was, the more drastically the germinability is reduced. Our data indicate that the phenotypic effect of gamma rays is stronger than the DMS effect.

The highest frequency of phenotypic changes was noticed with 150 Gy gamma irradiation (42.9%); the frequency of phenotypic changes after DMS treatment was only 27–28%. The plant development was delayed and was followed by death. Among the DMS-treated plants, there were no such phenomena; therefore the used concentrations of this chemical are not lethal (**Table 1**).

Mutagenic factors affect biochemical processes in seeds, impairing metabolism and causing unnatural changes, which in its turn influences vital processes in seeds and plants emerging from them. Therefore, studies of microspore formation (meiosis) are a reliable way to investigate the genetic variability of organisms at the cellular level and to evaluate effects of mutagenic factors on chromosomes of pollen mother cells (PMC) of sunflower lines.

Mutagen	Concentration (%) /dose (Gy)	Number of treated seeds	Number of plants from treated seeds	Number of phenotypically unchanged plants	Number of phenotypically changed plants	Phenotypic effect (%)
Control	No treatment	250	220	220	0	0.0
DMS	0.01	250	218	157	61	28.0
	0.05	250	208	152	56	27.0
Gamma rays	120	250	38	22	16	42.1
	150	250	28	16	12	42.9

Table 1.
Phenotype effect in the M₁ generation of sunflower (average across lines).

Microspores in anthers of flowering plants are the final result of meiosis, which can be traced on temporary and permanent microslides made from immature anthers.

The genotypes of the new self-pollinated lines—sterility fixers (Kh1002B and Kh1008B) and lines— pollen fertility restorers (Kh06134V and Kh201V) pre-treated with chemical mutagen DMS at concentrations of 0.01 and 0.05% or gamma-irradiated at doses of 120 and 150 Gy were studied.

The steps of microslide preparation to investigate chromosomes in sunflower meiosis were as follows:

1. Collection of specimens in the field—calathidium segments (d = 2–3 cm) with anthers. Green star phase.
2. Fixation of the specimens in Clark’s solution (absolute alcohol/glacial acetic acid 3:1) for 24 h.
3. Washing out the specimens in 70% ethanol until the odor of acetic acid disappears.
4. Storage of the specimens in 70% ethanol.
5. Staining the specimens in 2% aceto-orcein for 12–24 h. Aceto-orcein solution was prepared as follows: dissolve 1 g of dye in 45 ml of glacial acetic acid and 55 ml of distilled water. Dissolution is carried out in a reflux flask in a water bath for 30–60 min. After cooling, the solution of aceto-orcein is filtered and placed in a glass stoppered bottle. As a part of the study, we demonstrated that aceto-orcein was more effective for staining sunflower chromosomes than acetocarmine.
6. The stained specimen is placed on a mount in a drop of 45% acetic acid or in a drop of 0.5% aceto-orcein, covered with a cover slip and heated above an alcohol burner until boiling.
7. The slide is carefully crushed with a match to get a cell monolayer under the glass and examined under a microscope.

Meiosis was examined under a Micromed XS-5520 microscope at magnification of 40× and 100×. Oil immersion (special immersion oil, cedar oil, or glycerol)

was used to study slides at magnification of 100×. To document and illustrate the results, microphotographs were taken with a Nikon D 3200 kit VR camera equipped with a special Asian Microscope Adapter.

Cells with meiosis disorders were counted by metaphase-anaphase method: the percentage of cells with abnormalities was calculated related to the total number of cells under examination.

Analysis of meiosis in archisporial cells showed considerable effects of DMS and gamma rays on chromosomes in the M_1 , which manifested themselves as occurrence of significant chromosomal aberrations compared to the control ($P < 0.99$). The effect level depended on the mutagen exposure.

For example, after DMS treatment, the percentage of cells with abnormalities ranged within 7–14% (0.01%) and 12–20% (0.05%), significantly exceeding the control. After gamma irradiation, the percentage of cells with abnormalities ranged within 16–19% (120 Gy) and 20–25% (150 Gy), significantly exceeding the control.

Comparison of the results showed that the effect of gamma rays on meiosis of the lines under investigation significantly differed ($P < 0.99$) from that of DMS. Gamma rays resulted in the occurrence of more abnormal tetrads in the M_1 compared to DMS treatment. After irradiation, the percentage of abnormal tetrads ranged from 16.00% in line Kh1008B (120 Gy) to 27.10% in line Kh201V (150 Gy), whereas in DMS - treated lines, the percentage of abnormal tetrads ranged from 1.55% in line Kh201V (0.01%) to 21.65% in line Kh1008B (0.05%).

We observed normalization of meiosis and elimination of cells with abnormalities in subsequent mutant generations of sunflower compared to the M_1 .

In line Kh06134V, the percentage of cells with abnormalities in different phases of meiosis in the M_2 varied within 8.09–8.69% (0.01 and 0.05% DMS) and within 5.96–8.16% (120 and 150 Gy gamma irradiation). In the M_3 , the percentage of aberrations varied within 3.36–4.09% after 0.01 and 0.05% DMS treatment and within 4.29–5.34% after 120 and 150 Gy gamma irradiation.

In line Kh201V, the percentage of cells with abnormalities in the M_2 varied within 4.53–8.45% after DMS treatment and within 7.79–9.48% after gamma irradiation. In the M_3 , these values were 2.54–4.96 and 2.15–3.48%, respectively.

In line Kh1002B, the percentage of cells with abnormalities in the M_2 varied within 6.06–4.89% after DMS treatment and within 6.91–7.44% after gamma irradiation. In the M_3 , these values were 3.35–4.66 and 3.60–4.83%, respectively.

In line Kh1008B, we noted 4.92–6.95% of cells with abnormalities in the M_2 after DMS treatment and 6.42–10.77% after gamma irradiation. In the M_3 , these values were 2.15–3.57 and 3.09–5.26%, respectively (**Figure 1**).

The identified meiotic abnormalities in mutants were manifested as a chromosome lag during the formation of metaphase plate, impaired chromosome distribution in metaphase II, distorted metaphase plates, a chromosome lag in anaphase, asynchronous division during the second stage of meiosis, formation of pentads, triads, dyads, etc. (**Figure 2**).

Note. 1, outsider chromosomes in anaphase I; 2, asynchronous division during the second stage of meiosis; 3, chromosomes outside the metaphase plate in metaphase I; 4, abnormal tetrads.

All the specimens had phenotypic alterations (bent stem, dwarfism, absence of generative organs, chlorophyll deficit, deformation of generative organs, etc.) during subsequent development (**Figure 3**).

To prevent cross-pollination between different sunflower lines, individual inflorescences had been isolated the day before semiflorets opened, the offspring of which were to be examined the next year as the M_2 families. Concurrently, controls, non-treated with mutagens lines, were isolated.

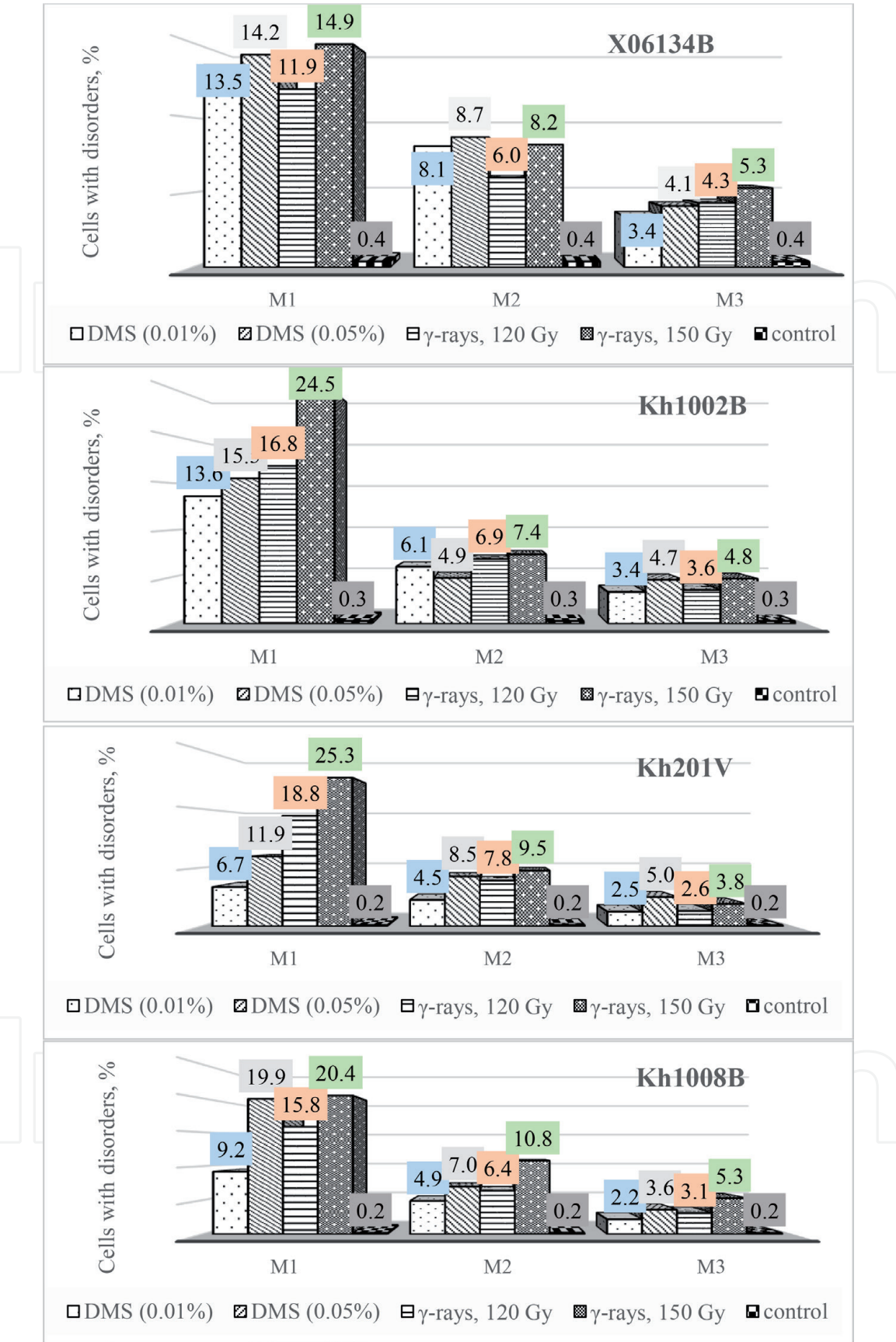


Figure 1.
Normalization of meiosis and gradual attenuation of the mutagenic effects of DMS and gamma rays in the M_1 – M_3 sunflower (%).

During the vegetation period, phenological observations of the growth and development of mutant plants were conducted; the field germinability was determined; cytological analysis was performed; and biometric measurements were

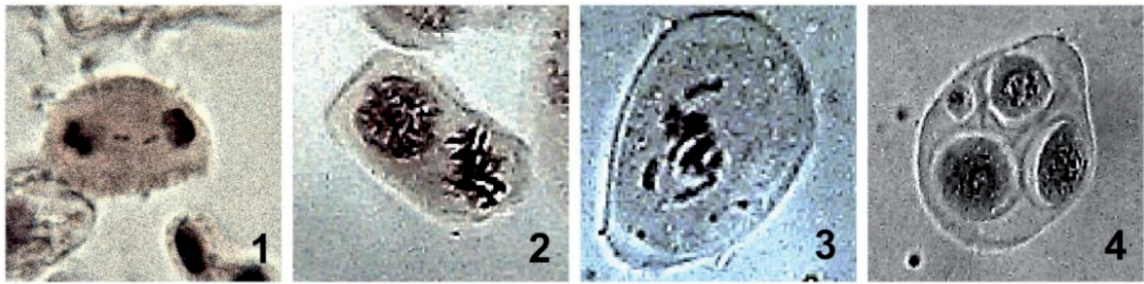


Figure 2.
Microphotographs of meiotic abnormalities in mutant generations of self-pollinated sunflower lines.

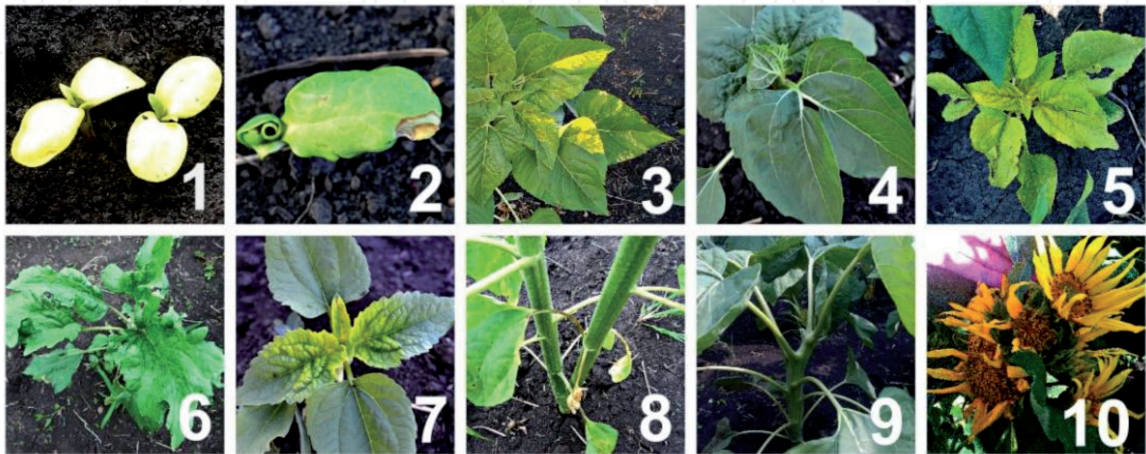


Figure 3.
Phenotypic effects of chemical and physical mutagens in the M_1 sunflower. 1, chlorophyll-deficient shoots from gamma-irradiated seeds (150 Gy); 2, 4, 6, morphoses induced by DMS (0.05%) in the early stages of plant development; 3, 5, 7, chlorophyll morphoses induced by DMS (0.05%); 10, deformation of generative organs induced by DMS (0.01%); 8, stem fasciation induced by DMS (0.05%); 9, absence of generative organs induced by gamma irradiation (120 Gy).



Figure 4.
Morphological changes observed in the M_1 of self-pollinated line Kho6134V: 1, xantha chlorophyll mutation 'golden tip' (0.05% DMS), and 2, "purple tint of leaves" mutation (0.01% DMS).

made (plant height measured 20 days after anthesis, calathidium diameter, and number of leaves per plant). Mutant plants were evaluated for the following parameters: oil content (%), 1000-seed weight, and fatty acid composition of oil.

In the M_1 , there were a lot of plants with different phenotypic developmental defects compared to the controls. However, one should keep in mind that most of them were so-called morphoses and consequences of phenotypic variability; such changes are not inherited and disappear in M_2 .

It is impossible to detect recessive mutations in M_1 plants, since of 2 alleles of a gene, as a rule, one allele only mutates, and the altered recessive allele is always

paired with the unchanged dominant allele (AA–Aa); therefore, selection of mutations is started with M_2 .

Only dominant mutations found by some researchers in some crops (usually in wheat) after exposure to chemical supermutagens can be detected in M_1 . Thus, examining the M_1 of self-pollinated line Kh06134V, we distinguished some morphological changes: a chlorophyll-deficient mutation (xantha) called “golden top” (0.01% DMS) and a mutation of a purple tint of leaves (0.05% DMS), which is stably expressed in subsequent mutant generations (**Figure 4**).

5. M_2 and M_3 generations, investigation, and use of mutations

M_2 is sown by families and single plants or by continuous sowing according to variants of mutagenic treatment, with optimal convenient density.

Plants were selected in the second mutant generation by visible morphological and physiological alterations to obtain macromutants as well as well-developed plants without visible alterations to find biochemical mutations and micromutations of quantitative traits. In addition, seeds from families without changes in the M_2 were sampled to reveal them in the M_3 .

Analysis of the mutant frequency in the M_2 showed that gamma rays (120 and 150 Gy) produced more plants with alterations than DMS (0.01 and 0.05%). The percentage of plants with alterations after gamma irradiation ranged 36.0–36.4%, while the percentage of plants with alterations after DMS treatment was within 9.6–9.8%. We noted the individual genotypic responses of the lines to the increase in the concentration and dose of mutagens. The rise in the number of plants with alterations in the M_2 depended on the increase in the concentration of DMS and the dose of gamma rays (**Table 2**).

In particular, in line Kh1002B, the total frequency of alterations was 3.2% with 0.01% DMS and 3.5% with 0.05% DMS, whereas plants with alterations were much more numerous with gamma rays (120 Gy–22.6%, 150 Gy–27.8%) (**Table 2**).

0.01% DMS-treated line Kh06134V gave the total frequency of plants with alterations of 14.7%, and the total frequency of plants with alterations after 0.05% DMS treatment was 10.0%. Gamma irradiation produced significantly more plants with alterations: 120 Gy produced 36.6% of plants with alterations, and 150 Gy–47.5% (**Table 2**).

In line Kh1334V, the total frequency of plants with alterations was 3.4 and 3.3% with 0.01 and 0.05% DMS, respectively (**Table 2**).

0.01 and 0.05% DMS produced 8.9 and 13.1% of plants with alterations, respectively, in line Kh201V. However, the effect of gamma rays was more conspicuous, and the total frequency of plants with alterations was 32.1 and 37.5% after 120 and 150 Gy exposure, respectively (**Table 2**).

Most of the alterations observed in the M_2 of the gamma-irradiated lines were nonheritable modifications found in early stages of the plant development, which disappeared during growth, whereas most of the DMS-induced alterations detected in different stages of the plant development were stable. The mutation nature of the changes in the M_2 was finally established by inheritance in the M_3 families.

We studied inheritance of mutant traits in the M_3 and subsequent generations. We also assessed the new mutant lines for breeding value and tested them for economically valuable traits, intending to involve constant valuable forms in hybridization and heterosis breeding in order to obtain new sunflower hybrids.

Having evaluated the alterations, we identified mutants noticeable for oil content in seeds, fatty acid composition of oil, 1000-seed weight, and resistance to the pathogen of sunflower downy mildew.

Original line	Mutagen concentration/ dose	The total frequency of mutations (%)	Frequency of chlorophyll mutations (%)	Frequency of morphological mutations (%)	Frequency of economically valuable mutations (%)
Kh1002B	0.01% DMS	3.2	0.6	1.5	1.13
	0.05% DMS	3.5	0.9	2.1	0.6
	120 Gy γ -rays	22.6	10.7*	7.1*	4.8
	150 Gy γ -rays	27.8*	2.8	13.9	11.1*
	LSD 05	1.5	0.8	1.0	0.8
Kh06134V	0.01% DMS	10.0	1.9	3.3	4.7
	0.05% DMS	14.7*	3.5	5.9	5.3
	120 Gy γ -rays	36.6	3.3	20.0	13.3*
	150 Gy γ -rays	47.5*	16.4*	21.3	9.8
	LSD 05	4.6	2.6	3.3	3.0
Kh1334V	0.01% DMS	3.4	0.6	1.5	1.3
	0.05% DMS	3.3	1.7*	0.7	0.9
	LSD 05	1.4	0.8	0.8	0.8
Kh201V	0.01% DMS	8.9	2.0	4.1	2.9
	0.05% DMS	13.1*	4.4*	5.0	3.7
	120 Gy γ -rays	32.1	5.2	18.7	8.2
	150 Gy γ -rays	37.5*	8.6*	18.4	10.5*
	LSD 05	4.1	2.2	3.1	1.6
Average across 12 lines					
DMS	0.01%	9.6	3.1	3.7	2.8
	0.05%	9.8	3.2	3.8	2.8
γ -rays	120 Gy	36.0	11.3	16.3	8.3
	150 Gy	36.4	8.2	18.9	9.3

* Difference significant at LSD 05.

Table 2.
Relative frequencies of major mutations induced by DMS and gamma rays in the M₂ sunflower, % (exemplified by 4 lines).

The traits of 1000-seed weight and oil content in seeds followed different patterns, depending on the genotypes of the self-pollinated lines. Thus, in the M₃ there were genotypes, in which DMS treatment and gamma irradiation increased the content of oil in seeds (Od973B, Kh1002B, Mkh845B, X0816B, Kh06135V, Kh1334V, and Kh201V) and 1000-seed weight (Kh808B, Kh1002B, Mkh845B, Kh0816V, Kh785V, Kh1334V, and Kh201V). On the whole, 1000-seed weight insignificantly varied in the M₃ (2–10%).

The mutants with increased content of oil in seeds are listed below: Kh1002B No 224 (0.05% DMS), 50%, and No 876 (150 Gy gamma rays), 48% (46% in the control); Kh0816V No. 422 (0.01% DMS), 50% (53% in the control); Kh1334V No. 609 (0.01% DMS), 48%, and No. 658 (0.05% DMS), 46% (43% in the control); and Kh201V No 685 (0.01% DMS), 54%, and No 1143 (150 Gy gamma rays), 52% (48% in the control) (**Figure 5**).

The mutants with increased 1000-seed weight are listed below: Mkh845B No. 385 (0.05% DMS), 64 g; No. 996 (150 Gy gamma rays), 67 g (48 g in the control);

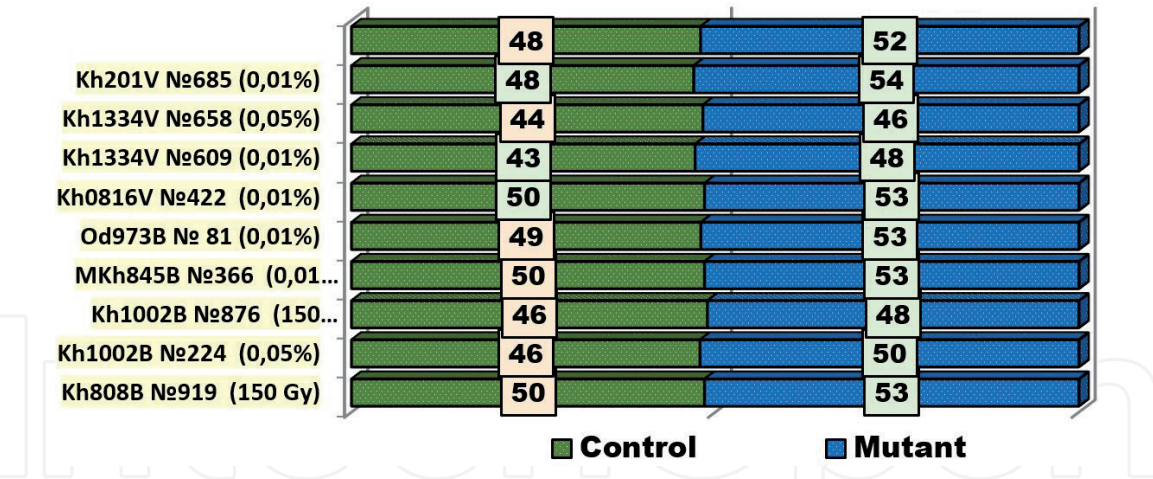


Figure 5.
Oil content in seeds of the mutant families (%).

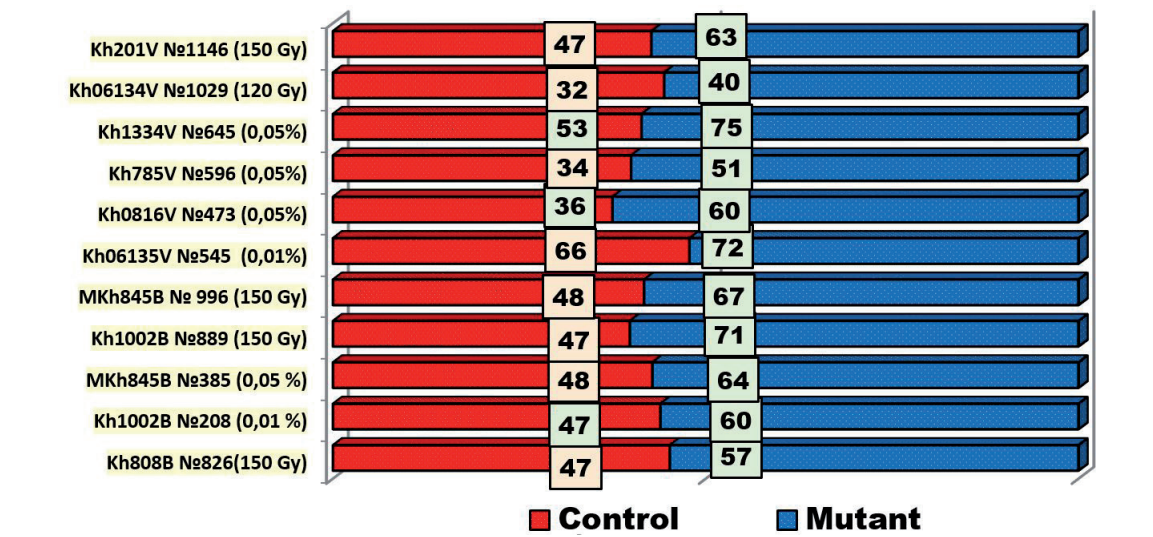


Figure 6.
1000-seed weight in the mutant families (g).

Kh06134V No. 1029 (120 Gy gamma rays), 40 g (32 g in the control); Kh785V No. 596 (0.05% DMS), 51 g (34 g in the control); Kh1334V No. 645 (0.05% DMS), 75 g (53 g in the control); and Kh201V No 1146 (150 Gy gamma rays), 63 g (47.1 g in the control) (Figure 6).

Biochemical analysis of oil from mutant sunflower seeds highlighted plants with increased content of linoleic acid of up to 70% (63% in the control) from line Kh201V. Among the mutants obtained from line Kh1334V, there were DMS-induced variants with increased contents of oleic and behenic acids (0.85% vs. 0.64% in the control), and such a combination is valuable for breeding (Table 3).

6. Conclusions

As exemplified by the M₁–M₃ mutant generations of sunflower, an important scientific challenge of determining peculiarities of the variability of quantitative and qualitative traits under the influence of DMS (0.01 and 0.05%) and gamma rays (120 and 150 Gy) was theoretically described, and a new solution to it was suggested. The frequency and range of mutational variability in the M₂ were summarized, and the inheritance of the mutant traits in subsequent generations was established. Chromosomal abnormalities in meiosis were characterized, and the

Original line	Mutant	Mutagen, (concentration/dose)	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Behenic
			C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C22:0
Kh201V		Control	6.67	0.47	3.87	25.34	62.75	0.28	0.24
	№742	DMS, 0.05%	6.40	0.41	4.95	16.72	70.79	0.15	0.37
	№694	DMS, 0.01%	6.71	0.56	4.00	25.34	62.85	0.13	0.21
	№1133	γ-rays, 120 Gy	7.25	0.80	3.47	17.55	70.54	0.12	0.11
Kh1334V		Control	3.43	0.11	3.78	87.28	3.51	0.35	0.64
	№659	DMS, 0.05%	3.29	0.11	3.54	89.10	2.00	0.30	0.85
	№642	DMS, 0.05%	3.71	0.12	3.52	88.48	2.15	0.30	0.85
	№628	DMS, 0.01%	3.83	0.17	3.85	87.25	2.75	0.47	0.84
	№609	DMS, 0.01%	3.54	0.15	3.40	86.90	3.92	0.35	0.83

Table 3.
Fatty acid composition of oil from the M₃ sunflower seeds.

breeding-genetic values of induced mutations as well as possibilities of their use in breeding were evaluated. Methodological peculiarities of the mutational breeding of sunflower as a cross-pollinated crop were defined, and new mutants with changed features were detected. In addition, this study allowed us to conclude that DMS was more effective than gamma rays for the induction of valuable for breeding mutations in new homozygous self-pollinated sunflower lines.

Thus, induced mutagenesis is a major component of the complex breeding process of creation of new parental lines and hybrids of sunflower with economically valuable characteristics.

Author details


Victoria Mykhailenko^{1*}, Viktor Kyrychenko², Alexander Bragin¹
and Dmitry Chuiko¹

¹ Department of Genetics, Selection and Seed Production, Faculty of Agronomy, Kharkiv National Agrarian University Named after V. V. Dokuchayev, Kharkiv, Ukraine

² Plant Production Institute Named after V. Ya. Yuryev of NAAS of Ukraine, Kharkiv, Ukraine

*Address all correspondence to: toryvasko@gmail.com

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