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Induced Mutagenesis for the Creation of New Starting Material in Sunflower Breeding

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Abstract: The article colligates data of studies on the variability of quantitative and qualitative traits in mutant sunflower M_1-M_3 generations affected by dimethyl sulfate (DMS) (0.01, 0.05 %) and gamma rays (120; 150 Gy), frequencies and range of mutations in M_2 and their inheritance in mutant families, chromosome aberrations in meiosis, as well as on the breeding and genetic value of induced mutants and possibilities of their use in breeding. The methodical peculiarities of the mutational breeding of the cross-pollinating crop were defined, and new mutants with changed features were created.

Investigating new homozygous self-pollinated sunflower lines, we observed a more negative mutagenic impact of gamma irradiation (120 and 150 Gy) on the germinability of M_1 sunflower seeds in the field compared with the DMS effect (0.01 and 0.05 %). The field germinability of DMS-treated seeds was 83–87 % vs. 11–15 % of gamma-irradiated ones.

The mutagenic effect of gamma rays (120 and 150 Gy) on M_1 meiosis was shown to be stronger than that of DMS (0.01 and 0.05 %). The percentage of cells with alterations varied within 15.79–18.78 % (120 Gy) and 20.38–25.26 % (150 Gy) compared to 0–0.16 % in the control.

The effect of gamma rays on the frequency of morphoses in M1 was stronger, in particular, after exposure to 120 Gy or 150 Gy of gamma irradiation, the number of plants with alterations was 43 %, whereas after DMS treatment (0.01 and 0.05 %) this parameter averaged 27–28 %.

We determined the inheritance of mutations of quantitative and qualitative traits, which are important for breeding, in mutant M2 families and selected mutant families with inherited altered traits that can be considered as mutations. Among the best mutations, there are morphological mutants with marker

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traits, mutants with increased content of oil in seeds, increased 1000-seed weight, increased contents of behenic, linoleic and palmitoleic acids as well as with absolute resistance to downy mildew.

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

Introduction

Sunflower is a leading crop in oil crop production in Ukraine. Therefore, the purpose of breeding is to combine high performance, oil content, group immunity to diseases and pests, adaptability to environmental conditions and high quality of products in one sunflower hybrid. Creation of such hybrids is possible only if there is diverse starting material with high capacity to pass quality traits to offspring and if specific breeding methods are developed.

For successful solutions to these challenges, it is important to have new starting material. Induced mutagenesis is a way to create starting material. This method increases the variability of morpho-biological traits in plants and induces mutations that can boost the expression of totally new valuable traits, which were previously unknown in breeding. This would accelerate the breeding process.

Induced mutagenesis is useful to widen the genetic diversity of self-pollinated sunflower lines, to increase the frequency and range of mutations and to create new starting material for breeding.

Owing to induced mutagenesis in sunflower breeding, certain successes were achieved: researchers obtained sunflower mutants with increased content of the most valuable oleic acid (up to 90 %), which became parents of high-oleic hybrids combining high yields, high oleic acid content and group resistance to major pathogens; the genetic diversity of the crop was widened; forms with a modified morphotype (plant height, calathidium diameter and shape, colour of leaves and ray flowers, growing season length etc.) were generated.

However, the effectiveness of mutagens in inducing valuable mutations in modern homozygous self-pollinated sunflower lines is not sufficiently studied, hence, at the current stage of studies, it was necessary to focus on increasing the effectiveness of creation of different mutants that could be valuable for genetic and breeding investigations as well as on searching for entirely new sources with high performance, yield capacity and resistance to biotic and abiotic factors. At the same time, as new homozygous lines appear, there is a need to study mutagens and microsporogenesis abnormities in M_1-M_2, the variability of quantitative and

qualitative traits, expression and frequency of morphological mutations in subsequent mutant generations.

Materials and methods

Induced mutagenesis was investigated at the Department of Genetics, Breeding and Seed Production of Kharkiv National Agrarian University named after V.V. Dokuchaev in 2014–2016. Our objective was to study the variability of quantitative and qualitative economic traits affected by dimethyl sulfate (DMS) and gamma irradiation and to evaluate the effectiveness of induced mutagenesis for the creation of starting material based on valuable mutations.

During the study, the meteorological conditions were characterized by unstable temperature and rainfall, which reflected zonal peculiarities in all the study years, however, the conditions generally were optimal for the growth and development of mutant sunflower plants.

Twelve different new homozygous self-pollinated sunflower lines bred at the Plant Production Institute named after V.Ya. Yuriev of NAAS (PPI nd. a. V.Ya. Yuriev) was used as the test material. Seeds of these lines were treated with mutagens.

In the gamma-ray experiments, dry seeds were once gamma irradiated (120 or 150 Gy) with a cobalt unit (remote gamma-ray unit Theratton-Elit-80; irradiation source: ^{60}Co) at Kharkiv Regional Oncology Center, Department of Radiation Therapy, Laboratory of Gamma irradiation.

To study the influence of chemical mutagens, supermutagen dimethyl sulfate at concentrations of 0.01 % and 0.05 % was used. This substance belongs to the most widespread and most effective group of chemical mutagens – alkylating compounds. Their act via alkylation of DNA, adding methyl, ethyl, amine and other groups to it.

Seeds of sunflower lines were treated with aqueous solution of chemical mutagen in compliance with a conventional method (Zoz, 1968). The exposure was 18 hours, which according to Artemchuk and Lohvynenko (2003), and Artemchuk (2007) is optimal for this group of mutagens. Seeds non-treated with mutagens served as the control.

Gamma irradiated or treated with chemical mutagen seeds were sown on separate plots of 20 m^2 (one-row plots, 25 plants per plot) in 2014; on plots of 40 m^2 (one-row plots, 25 plants per plot) in 2015; and on plots of 50 m^2 (one-row plots, 25 plants per plot) in 2016. The sowing arrangements were 70 × 25 cm. Seeds were planted with manual planters within the optimal timeframe (2nd - 3rd ten

days of May), The forecrop was winter wheat. Mutant plants in the experimental plots were cut and threshed manually.

M_1 plants were harvested, threshed and subsequently sown separately. All the M_2 families originated from M_1 plants were plants of the same type. Not many changes in M_2 plants were hereditary. Therefore, we verified the inheritance of the traits observed in M_2. For this, the altered specimens selected in M_2 were sown as families in M_3. Analysis of M_3 allowed us to determine, in addition to the nature of mutations, the type of their inheritance.

To determine the nature of morphological changes caused by chemical and physical mutagens, we studied the microspore formation on temporary slides prepared from young anthers.

To study meiosis, calathidium segments (d = 2–3 cm) with anthers were separated and fixed in ethanol: acetic acid mixture (3:1) for 24 hours. Then they were washed out three times in ethanol and stored in 70% ethanol at +4 °C. Chromosomes of pollen mother cells were stained with 2% aceto-orcein for 12 hours. We found that sunflower anthers were better stained with aceto-orcein than with acetocarmine.

Meiosis was studied in samples squashed in 40% acetic acid on temporary slides. The procedure of preparation of squash temporary slides was as follows: anthers were removed from flowers of mutant plants with tweezers; all further operations were carried out on a slide in a drop of 0.5% aceto-orcein or in 40% acetic acid in order to prevent the slide drying; anthers were placed on a slide and crushed with preparation needles to release microsporocytes from the pollen sac; the remnants of anther cuticle were removed; the slide was covered with a coverslip and warmed over an alcohol burner until boiling began; the sample was squashed with a match in order to make a one-cell layer under the cover-slip.

To evaluate the effects of mutagens on meiosis in sunflower cells, the metaphase-anaphase method was used: the total number of cells in these phases and the number of normal tetrades were calculated, and the percentages of cells with aberrations related to the total number and for each phase were computed (Pausheva, 1988).

Meiosis was investigated under a microscope Micromed XS-5520 using magnification 40 × and 100 × . Magnification of 100 × was augmented with oil immersion (special immersion oil, cedar oil, or glycerin). To document the data and to illustrate the results, microphotographs were taken using a camera Nikon D3200 kit VR equipped with a special microscope adapter.

Phenological observations were performed during the growing season; the growth and development of mutant plants were monitored; the field germinability was recorded; and the plant height, calathidium diameter and a number

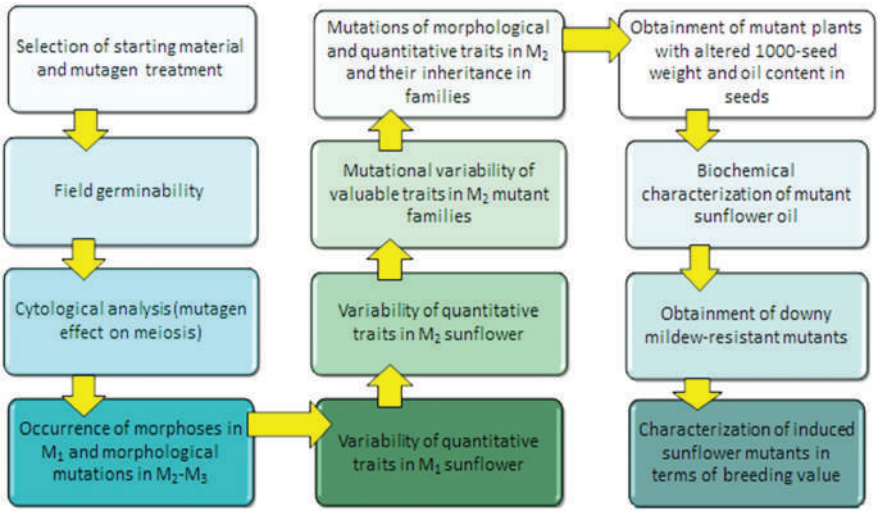

Figure 1: The stages of studying homozygous sunflower lines affected by DMS and gamma-rays, 2014–2016.

of leaves per plant were measured. Sunflower mutants were characterized in M_3 by morpho-biological traits, oil content in seeds, 1000-seed weight, the fatty acid composition of oil, and resistance to downy mildew (Figure 1).

In the winter, accessions were assessed for resistance to race 730 of the downy mildew pathogen in the Laboratory of Plant Immunity to Diseases and Pests. The express method developed in the PPI nd. a. VYa Yuriev was used for the assessment (Dolgova et al., 1990).

Lines were assessed by the alternative manifestation of resistance – "immune-susceptible". Assessing resistance of breeding accessions, we took conidial sporulation on cotyledon leaves and hypocotyls as a criterion of susceptibility. Plants without any signs of the disease were considered as immune (Borovska and Kolomatska, 2012). The resistance of sunflower accessions to the disease was determined by disease incidence and by percentage of affected plants related to the total number of plants under investigation (Chumakov *et al.*, 1974).

Results

Analyzing the field germinability of DMS-treated or gamma irradiated seeds of self-pollinated sunflower lines, we evaluated the effects of mutagens on plants,

which depended on the type and concentration/dose of mutagen. It was proved that gamma irradiation in doses of 120 and 150 Gy lowered the field germinability in comparison with the control and that the effect of gamma irradiation (120 and 150 Gy) on the germinability was more negative than the action of DMS at concentrations of 0.01 and 0.05 %. In particular, the germinability of DMS-treated seeds in M_1 ranged on average within 83–87 % (control - 85 %), and the germinability of gamma-irradiated seeds only averaged 11–15 %. However, in M_2-M_3, the field germinability of seeds after gamma irradiation was closer to the control value than in M_1. In M_2, the germinability of seeds after gamma irradiation averaged 63 % (120 Gy) and 65 % (150 Gy) vs. 80 % and 75 % after DMS treatment (0.01 % and 0.05 %, respectively). In M_3, the field germinability was high and averaged 91 % (0.01 % DMS), 85 % (0.05 % DMS), 92 % (gamma rays, 120 Gy), and 88 % (gamma rays, 150 Gy) (Figure 2).

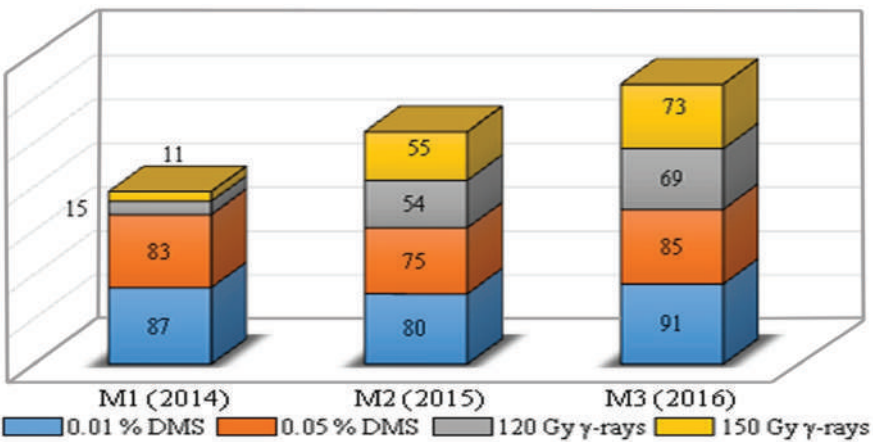

Figure 2: Influence of mutagens, DMS and gamma-rays, on the field germinability of sunflower seeds in M_1-M_3, 2014–2016 (average across the studied lines).

Assay of meiotic chromosomes in sunflower showed that despite their complete conjugation in prophase I, the subsequent stages of meiosis were distorted (Figure 3).

Assay of meiosis in archesporial cells of M_1 demonstrated strong mutagenic effects of DMS and gamma irradiation on meiosis in M_1 in comparison with control cells. The physical mutagen induced a greater number of cells with aberrations than the chemical one. The higher the dose of mutagen was, the greater the number of cells with aberrations became.

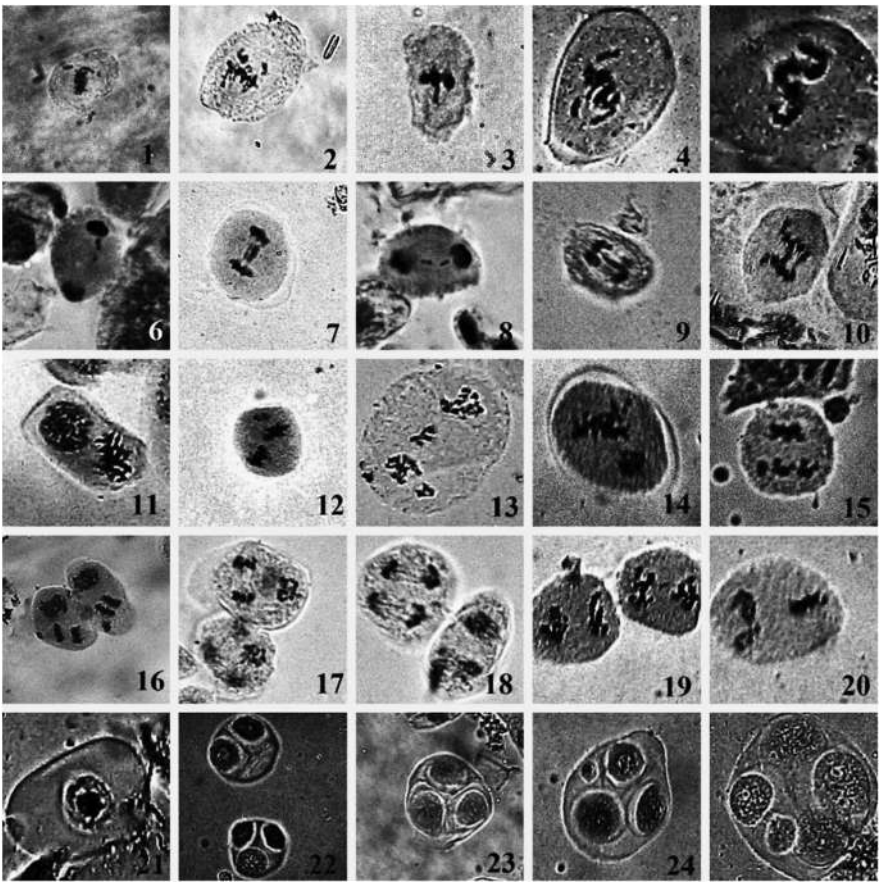

Figure 3: Microphotographs of meiosis in mutant sunflower generations affected by gamma-rays and DMS, 2014–2016.
1, 3 – chromosomes outside the metaphase plate in metaphase I (gamma-rays); 2, 4 – chromosomes outside the metaphase plate in metaphase I (DMS); 5 – deformation of the metaphase plate in metaphase I (DMS); 6 – lagging chromosomes in anaphase I (gamma-rays); 9 – lagging chromosome bridges in anaphase I (gamma-rays); 7, 10 – lagging chromosome bridges in anaphase I (DMS); 8 – lagging chromosomes in anaphase I; 11–13 – asynchrony in the metaphase plate formation and a chromosome bridge in metaphase II (DMS); 14, 15 - disorders in the metaphase plate formation in metaphase II (gamma-rays); 16, 20 – asymmetric chromosome disjunction to poles in anaphase II (DMS); 17–19 – disorders in anaphase II during chromosome disjunction to poles (gamma-rays); 21 – a monad (gamma-rays); 22 – a dyad and a triad (gamma-rays); 23 – a triad (DMS); 24 – a tetrad with a micronucleus (DMS); 25 – a pentad (DMS).

After DMS treatment, the percentage of cells with aberrations varied within 7–14 % (0.01 %) and 12–20 % (0.05 %), which significantly exceeded the control. The highest percentage of abnormal cells was detected in metaphase II: it ranged from 15 % to 56 %. The percentage of abnormal tetrades ranged from 1.5 % to 22 %.

After gamma irradiation, the percentage of cells with aberrations varied within 16–19 % (120 Gy) and 20–25 % (150 Gy), which significantly exceeded the control. The highest percentage of cells with meiotic distortions was also seen in metaphase II, ranging from 21 to 50 %.

In addition, gamma irradiation induced a greater number of aberrant tetrades in M_1 compared with DMS treatment. The percentage of aberrant tetrades varied within 7–27 % after gamma irradiation vs. 1.5–22 % after DMS treatment.

We found that in M_2-M_3, compared to M_1, the division of cells was mostly normal. A small percentage of cells with aberrations in M_3 indicated their elimination and normal meiosis in the test lines (Figure 4).

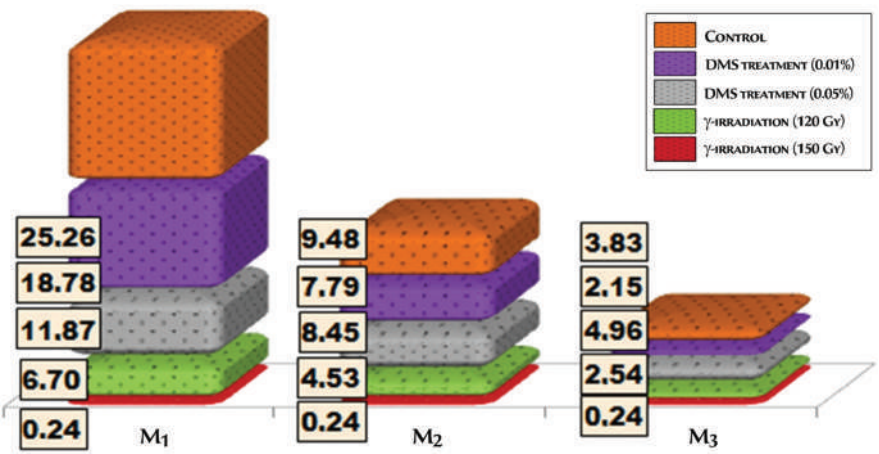

Figure 4: Effect of mutagens with gradual normalization of meiosis in M_3 sunflower, 2014–2016 (average across the studied lines) (*Footnote significant difference, P<0.99*).

In 2014, a wide range of morphoses in M_1 (Figure 5) was seen, and we found that their number was determined by the individual genotypic response of self-pollinated lines and the type of mutagen.

In particular, a stronger negative effect of the physical mutagen compared to the chemical one was observed: after gamma irradiation the number of plants with abnormalities varied within 15–68 % (120 Gy) and 24–72 % (150 Gy), while after DMS treatment it was 12–41 % (0.01 %) and 15–46 % (0.05 %).

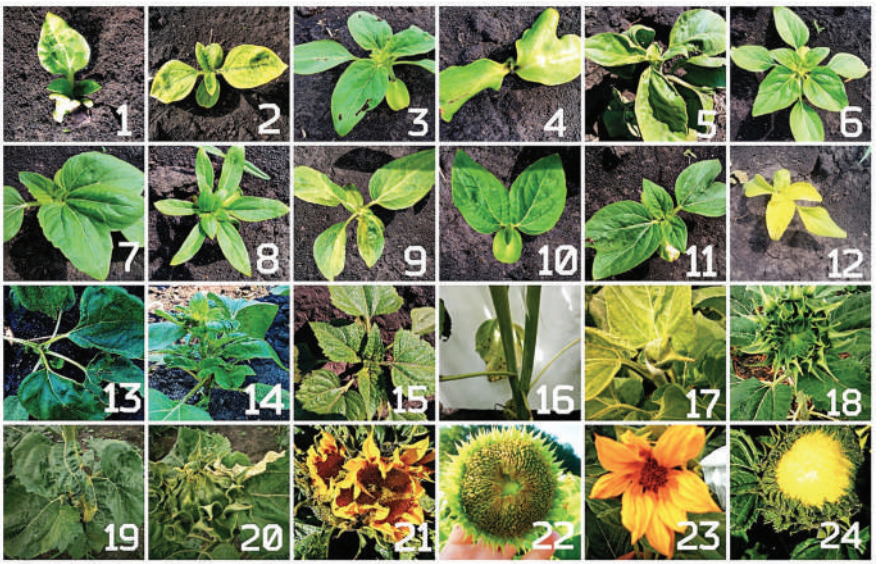

Figure 5: Gamma-Ray and DMS-Induced morphoses in M_1 of sunflower lines at different stages of the plant development, 2014: 1–17, 21, 23 – morphoses induced by 0.01 % or 0.05 % DMS; 18–20; 22, 24 – morphoses induced by 120 Gy or 150 Gy gamma-irradiation.

It was also found that gamma irradiation had a greater negative effect on the plant height and number of leaves than DMS treatment. The average height was 141–143 cm (DMS) *vs*. 113–125 cm (gamma irradiation), with the control height of 147 cm. The number of leaves was on average 27 in DMS-treated accessions and 17–20 in gamma-irradiated ones, with the control value of 27.

In M_1, there were morphoses of different types, from early phases of development to anthesis, associated with the size, shape and number of cotyledon leaves, color of leaves at the growth points, shapes and sizes of calathidium and ray flowers, plant habitus, venation, shape and number of leaves, fasciation of stems, leaves and calathidiums. Specifically, line Kh06134V had DMS-induced mutations: chlorophyll mutation *Xantha* called "golden apex" (0.01 % DMS) (Figure 6) and mutation of leaves with crimson tint (0.05 % DMC).

Given the high frequency of altered plants in M_1, a wide range of plants with abnormalities in M_2 was predicted.

We identified different types of abnormalities, both in gamma-irradiated and DMS-treated specimens: pigment mutations (*Viridis, Virescent, Xantha, Whitish*, crimson tint of leaves), mutations changing the color of ray flowers (a mutant with lemon ray flowers induced by 150 Gy gamma irradiation), calathidium shape

Figure 6: A Dominant chlorophyll mutation, xantha type "Golden top", induced by 0.05 % DMC, 2014.

and size, mutations of the plant habitus (induced by 0.01 % DMS), leaf venation (a mutant with dichotomous venation and a modified calathidium in line Od973B induced by 120 Gy gamma irradiation, Figure 7), shape and number of leaves (multi-leaf forms induced by 0.05 % DMS), plant height, stem and leaf fasciation.

Figure 7: A mutant plant with dichotomous leaf venation and a modified calathidium induced in the line of Od 973 B by 120 Gy gamma-irradiation, 2015.

In M_2, the effect of gamma irradiation on the number of abnormal plants was stronger than DMS effect. The percentage of abnormal plants after gamma irradiation was 36.0 % *vs.* approximately 10 % after DMS treatment. The number of DMS and gamma ray-induced chlorophyll mutations was 3 % and 8–11 %, respectively; the number of morphological mutations - about 4 % (DMS) and 16–19 % (gamma irradiation); the number of valuable economic mutations - about 3 % (DMS) and 8–9 % (gamma irradiation) (Table 1).

Hence, the variability of quantitative traits in M_2 proved the effectiveness of action of DMS and gamma rays on the genotypes of our new homozygous self-pollinated sunflower lines.

Table 1: Relative frequency of major mutations induced by mutagens in M_2 sunflower, %, 2015.

Initial line	Mutagen	Concentration/ dose	Total frequency of mutations, %	Chlorophyll mutations, %	Morphological mutations, %	Valuable economic mutations, %
Kh 808B	DMS	0.01 %	15.8	5.4	6.7	3.7
		0.05 %	14.3	5.0	5.7	3.6
	Gamma-rays	120 Gy	40.9	13.6	18.2	9.1
		150 Gy	61.9*	14.3	38.1*	9.5
	HIP_{05}		5.0	3.2	3.7	2.7
Kh06134V	DMS	0.01 %	10.0	1.9	3.3	4.7
		0.05 %	14.7*	3.5	5.9	5.3
	Gamma-rays	120 Gy	36.6	3.3	20.0	13.3*
		150 Gy	47.5*	16.4*	21.3	9.8
	HIP_{05}		4.6	2.6	3.3	3.0
Kh201V	DMS	0.01 %	8.9	2.0	4.1	2.9
		0.05 %	13.1*	4.4*	5.0	3.7
	Gamma-rays	120 Gy	32.1	5.2	18.7	8.2
		150 Gy	37.5*	8.6*	18.4	10.5*
	HIP_{05}		4.1	2.2	3.1	1.6
The average is 12 lines	DMS	0.01 %	9.6	3.1	3.7	2.8
		0.05 %	9.8	3.2	3.8	2.8
	Gamma-rays	120 Gy	36.0	11.3	16.3	8.3
		150 Gy	36.4	8.2	18.9	9.3

In 2016, the inheritance of mutations of M_2 plants by M_3 families was verified. In M_2, a wide variability of quantitative traits was detected. It was due to the individual response of the genotype to the mutagens and diversity of the test homozygous self-pollinated sunflower lines. In particular, the genotypes of lines Od973B, Mkh845B, Kh0816V, Kh06135V, Kh06134V, and Kh201V appeared to be more susceptible to DMS and gamma irradiation, since in M_3 families a high degree of inheritance of mutations of M_2 plants was observed. The variations of the quantitative traits in M_3, on the whole, were insignificant (2–10 %).

In M_2, mutations of morphological and quantitative valuable-for-breeding traits (calathidium diameter, plant height, leaf number) were described and their inheritance in M_3 families was determined.

New mutants with an increased 1000-seed weight were selected: for example, mutant 645 (75 g), mutant 473 (60 g), mutant 208 (67 g), which significantly exceeded the control accessions.

In M_3, mutants with increased content of oil in seeds were detected: for example, mutant 685 (52 %), mutant 609 (48.1 %), mutant 422 (54.4 %), which significantly exceeded the control accessions.

Mutants with increased content of linoleic acid, up to almost 70 %, were identified (63 % in the control).

In DMS-induced mutants derived from line Kh1334V, a high content of oleic acid in combination with a high content of behenic acid was recorded, namely 0.85 % (0.64 % in the control), which is valuable for breeding.

We selected 6 mutants with absolute resistance to downy mildew, which was induced by 0.01 % DMS and 150 Gy gamma irradiation. We also found that 0.01 % DMS was more effective for the induction of stable mutations of such type.

Thus, we developed mutants, which are the valuable starting material for breeding and for widening the genotypic diversity of sunflower (Table 2).

Discussion

As Berezina and Kuzina (1964) reported, the first data on pre-sowing irradiation of sunflower seeds with X-rays at a dose of 38–114 Gy using an aluminium filter were obtained in 1933. Back at that time, a stimulatory effect of this mutagen on the growth and development of sunflower was noticed (Berezina and Kuzina, 1964). However, there is evidence that the first attempts to influence plants using X-rays or ultraviolet rays were made by the Italian researcher Pirovano (Pirovano, 1922) as early as in 1922.

Table 2: Characterization of induced mutants of sunflower in terms of breeding value.

Initial line	Mutant	Major features	Mutagen	Photograph
Kh201V	KhNAU742V	Modified habitus; number of leaves = 198 (23 in the control); $d_{calathidium}$ = 21 cm (11 cm in the control); linoleic acid content = 70.54 % (62.75 % in the control)	DMS, 0.05 %	
Kh201V	KhNAU 1133 V (KhNAU 01)	Lemon ray flowers (orange in the control), height = 122 cm (140 cm in the control); linoleic acid content = 70.54 % (62.75 % in the control)	Gamma rays, 120 Gy	
Kh06134V	KhNAU 488 V (KhNAU 02)	The crimson tint of leaves; oleic acid content = 30.05 % (26.50 % in the control)	DMS,0.01 %	
Kh06134V	KhNAU 505 V (KhNAU 03)	Yellow apex; height = 124 cm (138 cm in the control)	DMS,0.05 %	
Od973B	KhNAU 553B	Height = 142 cm (151 cm in the control); behenic acid content = 0.80 % (0.30 % in the control)	DMS,0.05 %	

(*continued*)

Table 2: (*continued*)

Initial line	Mutant	Major features	Mutagen	Photograph
Kh06135V	KhNAU 63B	Height = 66 cm (158 cm in the control); lemon ray flowers (orange in the control)	DMS,0.01 %	

The main purpose of experimental mutagenesis in sunflower is to create starting material for breeding. In studies on induced mutagenesis, when a wide assortment of starting material was available, mutants that differ from the initial forms by content and qualitative composition of oil in seeds were developed. For example, Soldatov (1976) created dwarf mutants, early-season forms and genotypes with increased content of oleic acid.

A team of researchers from the US (Universities in Georgia and Oregon) and Germany reported that chemically induced dominant mutation *Ol* significantly increased the amount of oleic acid and correlated with reduced expression of a seed-specific oleoyl-phosphatidyl choline desaturase in sunflower seeds (Shuppert *et al.*, 2006).

X-irradiating dry seeds of an inbred line with normal content of palmitic acid (≈3 %) and high content of oleic acid (≈ 88 %), Fernandez-Martinez *et al.* (1997) created mutant CAS-12 with high contents of palmitic acid (≈30 %) and palmitoleic acid (≈7 %) without reducing content of oleic acid. Later, Velasco *et al.* (2008), using ethyl methanesulfonate, and Encheva *et al.* (2012), ultrasounding corcules, developed mutants with increased content of palmitic acid (5–29 %) in M_1 and M_2 generations.

Kyrychenko and Poviakalo (1988), Maklyak *et al.* (2009) and some other researchers of the PPI nd. a. VYa. Yuriev of NAAS, using mutagenesis, created over 97 mutant high-oleic (75–92 %), high-palmitic (25 %), low-palmitic and low-stearic (with the total amount of these acids not more than 8 %) forms.

Diseases and pests of plants, annually destroying a significant part of yields, cause significant damage to the national economy. Creation of resistant starting

material is one of the most promising and realistic ways to effectively protect plants against different pathogens. Recently, a lot of scientists have tried to solve this challenge. For example, in Bulgaria, forms that were resistant to *Orobanche cumana* were generated by radiation mutagenesis (Encheva, 2009; Encheva *et al.*, 2014, 2012). De Oliveira *et al.* (2004), having treated seeds with ethyl methanesulfonate, identified plants resistant to *Alternaria helianthiin* in M_3. Lofgren and Ramaraje-Lers (1982), having treated sunflower seeds with different doses of chemical supermutagens, found plants with resistance to sunflower rust in M_2 and M_3. There is evidence that sunflower mutants resistant to certain herbicides were generated by induced mutagenesis (Berville *et al.*, 1992; Sala *et al.*, 2008).

We proved the effectiveness of the chemical mutagen, DMS, at the concentration of 0.01% to induce sunflower mutants resistant to downy mildew.

Some researchers demonstrated prospects of induced mutagenesis for creation sunflower forms with new morphological features. Significant successes in enriching the sunflower gene pool via induced mutagenesis were achieved by Kalaydzhyan (1996, 1998). They described mutagenic effects on frequencies and ranges of chlorophyll and physiological mutations, growing season length, plant height, oil content in seeds, *etc.*, depending on the type and dose of mutagen (nitrosoethylurea -NEU, DMS) as well as on the treatment mode. In addition, Kalaydzhyan *et al.* (2007, 2009) built up a genetic collection comprising 150 mutants, which differed by one or several traits.

Jambhulkar and Joshua (1999) confirmed that 200 Gy of gamma rays was an effective way to generate chlorophyll and morphophysiological mutants of sunflower, for example, 200 Gy irradiation of seeds of cultivar Surya induced the greatest number of mutants; 27 morphological mutants in M_2 generation were identified. In 2001, Usatov *et al.* (2001) treated line 3629 with NEU and also got a number of chlorophyll mutants of sunflower.

Christov (1996) identified a number of mutants in M_1-M_5 generations. One of the mutants had modified leaves and leaf petioles.

Cvejić *et al.* (2015) irradiated self-pollinated lines with gamma rays, fast neutrons and treated with NEU. They identified numerous valuable mutants. In the same year, Chetan Kumar *et al.* (2015) demonstrated the dependence of the variability of quantitative traits on gamma irradiation doses (10 Gy, 15 Gy and 20 Gy) in M_1 of parent lines of known hybrids.

Fambrini and Pugliesi (1996) obtained a chlorotic apex That was manifested as yellow cotyledons and as the first pair of true leaves of green colour, which made the apex yellow.

Cvejic and Bado (2009) studied the effect of gamma rays (^{60}Co), fast neutrons and NEU on M_1 seeds of sunflower inbred lines and found that gamma

irradiation had the greatest mutational effect followed by fast neutrons and NEU in different doses.

The influence of different doses of NEU was studied by a team of scientists from India, France, Argentina, Ireland (Kumar *et al*., 2013).

Taking into account the conclusions drawn by several researchers and by Zoz (1966), on the effects of chemical mutagens on plants compared with physical ones, we compared the actions of gamma rays and DMS and revealed that there were differences in the assortments of mutations and that the chemical mutagen was more active in inducing a number of mutations in the new homozygous sunflower lines.

Some scientists investigated the influence of chemical and physical mutagens on mature and immature sunflower seeds. For example, Soroka (2013) treated immature achenes of a sunflower line with 0.02% NEU and found a wide range of changes in morphological and physiological characteristics of M_1 plants and a wide range of mutations in M_2. Similar studies were carried out by Vasin (2008) and Lyakh *et al*. (2005), who found a wide range of numerous mutations, and their frequency rose with an increase in mutagen concentration and exposure. Encheva *et al*. (2002) gamma irradiated isolated immature corcules (^{137}Cs) at the dose of 5 Gy and obtained mutants with considerable variability in the plant height, stem and calathidium diameters, oil content in seeds, and 1000-seed weight.

Several studies showed strong effects of chemical and physical mutagens on the germination and field germinability of sunflower seeds. For example, having gamma irradiated two genotypes of sunflower at various doses, Jagadeesan *et al*. (2008) concluded that the germinability of seeds and the percentage of survival of mutant plants in M_1 generation were reduced. In M_2 generation, the means of plant height, seed yield and oil content, as well as their variability, increased.

Vijayata and Navnath (2016) described strong mutagenic effects of chemical mutagens (NEU and sodium azide) and gamma rays on the germination and field germinability of seeds.

Many researchers showed that chemical and physical mutagens induced different chromosome aberrations in somatic and germ cells of sunflower, which were not accidental and were due to the specific action, concentration or dose of mutagens.

Škorić (2012) found that gamma irradiation was the most effective for induction of chromosome aberrations, and with increasing doses of gamma rays, the numbers of aberrant cells and the number aberrations per cell rose significantly.

Arslan *et al*. (2001) obtained a broad range of chromosome aberrations, using gamma irradiation of sunflower seeds at doses of 10, 20, 30, 40 and 50 Gy, and noticed the dependence of the frequency of abnormalities on the dose of irradiation.

Prabakaran and Jayakumar (2014) investigated the effects of different doses of gamma rays and different concentrations of NEU on meiosis in M_1 sunflower and found that the higher dose or concentration of mutagens was, the higher frequency of chromosome aberrations, was and that gamma rays had a stronger mutagenic effect on meiosis in comparison with NEU.

Biletskiy et al. (1975) found that NEU induced a large number of non-nuclear chlorophyll mutations in sunflower (40 %).

Taking into account potentials of induced mutagenesis, one should recognize that a lot of theoretical and practical issues of the mutational process remain unclear, despite the fact that today a sufficient amount of information has been accumulated. For example, NB Tomlekova (2010) in the research "View Induced Mutagenesis for Crop Improvement in Bulgaria" reviewed studies on induced mutagenesis and their application in plant breeding, including sunflower breeding, conducted at different Bulgarian agricultural research institutes during the last half-century. In a book edited by Martinez-Force *et al.* (2015), the whole section is devoted to achievements, problems and prospects of mutagenesis in sunflower.

Theoretical compilation of data obtained by us and other researchers suggests that the achieved level of knowledge allows us to really plan sunflower mutagenesis and gives grounds to recommend the mutational breeding of sunflower as an effective trend in genetic improvement of this crop.

Summing up the comparative analyses of the genetic effects of chemical and physical mutagenesis, one should recognize that most researchers prefer chemical mutagens. However, in studies on numerous crops, advantages of chemical mutagens over physical ones, especially in the induction of valuable-for-breeding mutations, have not been proven. Basing on our results, we believe that at present there is not yet enough convincing evidence that would justify ignoring gamma irradiation in the mutational breeding. On the contrary, its widespread use along with the most effective chemical mutagens will only help to draw more substantiated conclusions.

Conclusions

Thus, summing up our studies, we can conclude that DMS was more effective in inducing valuable-for-breeding mutations than gamma irradiation on the new homozygous self-pollinated sunflower lines.

This is attributed to the fact that DMS is one of the most effective chemical mutagens, and the mechanism of its action is based on deep penetration of specific

agents in the organism and their effects on the gene structure. Despite the fact that the source of gamma rays used was a cobalt isotope, which is one of the best irradiators causing a large number of aberrations, most of them were not hereditary.

We cytologically studied meiotic chromosomes to confirm the effects of DMS and gamma irradiation on mutant generations of sunflower, which served as proof of the trueness of the mutants selected.

The mutants with modified quantitative and qualitative traits were selected as valuable starting material for breeding: morphological mutants, mutants with increased content of oil in seeds, with increased 1000-seed weight, with increased contents of behenic, linoleic and palmitoleic acids, and with absolute resistance to downy mildew.

Three mutant lines – pollen fertility restorers, which are a basis for the creation of highly heterotic hybrids, were registered with the National Center for Genetic Resources of Ukraine.

The expediency of using the new homozygous self-pollinated lines for efficient development of mutants with a set of modified values of useful economic features, which will contribute to widening the diversity of starting material for heterotic sunflower breeding, was rationalized.

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