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## Heritable variability in winter wheat at the interaction of genotype with factors of high genetic activity

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**Abstract.** Identification of new opportunities in the use of certain ecogenetic factors for the genetic improvement of winter wheat is a vital component of improving existing cereal agrocenoses within the framework of the food security strategy. The purpose of this study was to demonstrate the potential of substances with prominent genetic activity in inducing potentially beneficial changes and to establish the possibility of obtaining optimised schemes for obtaining new forms and more controlled trait variability. Mutations were detected by visual observation and biometric and biochemical analysis from the second generation to the sixth, with the inheritance of the detected traits in subsequent generations being checked. The study presented the possibilities of the mutation process for certain genetic and breeding-value forms, the specific features of induction of such traits, which makes it possible to increase the predictability of obtaining the necessary mutant lines and partially controlled high variability in certain traits. It was found that the most promising for use is the complex application of moderate doses of sodium azide and dimethyl sulphate as ecogenetic factors. It was shown that in combination with three varieties among the ones under study (Kalanča, Polyanka, Pochayna), it is possible to create highly efficient genotype-mutagenic systems with increased yield of valuable forms. The increase in mutagenic depression using more harmful substances can be substantially offset by an increase in the proportion of beneficial changes. The negative side is their complex nature. The nature of the ecogenetic factor is no less significant than the concentration of the

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mutagen or the genotype of the original form, which is sometimes overlooked. It was confirmed that the use of these factors is quite promising in terms of obtaining small biochemical changes (micromutations). Several promising mutant lines were identified that have a comprehensive improvement in grain yield and quality without additional negative changes. The findings of this study can be used in breeding programmes to create new varieties of winter wheat

**Keywords:** mutations; chemical mutagenesis; supermutagens; yield; grain quality

## INTRODUCTION

Changes in the environment require agricultural crops to adapt. The relevance of genetic improvement methods, such as experimental mutagenesis, is to create more resistant and adapted plant varieties that can survive in the face of climate change. Chemical mutagens have shown particular effectiveness in this regard. The effect of chemical mutagenesis on cultivated plants can cause mutations in their genome (Lal *et al.*, 2020), which can lead to heritable changes. According to J. Chaudhary *et al.* (2019), these mutations may be random and not always beneficial, but in some cases, they can lead to beneficial changes. The mutation process is random, and most mutations will not be beneficial or may be harmful to the plant. Therefore, after mutagenesis, the resulting plants are selected and tested to identify those with desirable properties, such as increased yields, disease resistance (Bezie *et al.*, 2020) or other useful characteristics. Thus, W. Yali and T. Mitiku (2022) found that the effect of chemical mutagenesis on cultivated plants can change their development and heredity, but the results will depend on the particular mutations that occurred and the characteristics they affected.

Chemical supermutagens with prominent damage capacity are one of the most popular factors for creating new mutant forms, especially with complex changes. They are used not only for genetic improvement of cultivated plants but also for research on the effect of mutagens on plant organisms in general to identify genetic control of traits. M. Spencer-Lopes *et al.* (2018) found that most of the successful production of complex economically valuable forms of major crops is conditioned by the effect of chemical mutagenesis, which, given the level of relevant biological research, is almost widely used to enhance the crossing components of parental forms, to investigate genetic systems for individual valuable key traits, to obtain fundamentally new biochemical compositions of traits (Kartseva *et al.*, 2023) in combination with other positive changes.

The site-specificity of chemical factors determines their increased affinity for certain structural features of cultivated plant genotypes, which can lead to a substantial increase in the yield of valuable forms. The researcher can identify and exploit these site-specific interactions, which can improve the efficiency of the breeding process by several orders of magnitude. G. Abaza *et al.* (2020) found that two key components must be considered: the variety as a subject for mutagenic action and the nature

of the chemical mutagenic factor. For factors of another nature, such variants are not possible. The resulting systems can generate 60-80% more moulds with positive changes. This approach requires a large volume of experiments with numerous variants of chemical supermutagens and concentrations, a large set of different varieties, preferably considering the ecological and geographical diversity (Ram *et al.*, 2019; Abdel-Hamed *et al.*, 2021) in origin. Changes caused by prominent concentrations of mutagens can be difficult to predict and control (Ahumada-Flores *et al.*, 2021). Different plants can react differently to the same mutagen, and this can complicate the breeding process.

The results of global statistics may vary depending on the plant species, particular mutagen, research conditions, and other factors. Therefore, it is vital to conduct research and consider all aspects before using high concentrations and highly damaging mutagens in genetic improvement. A more careful and controlled approach may be safer and more effective in achieving the desired results in crop plant breeding (Cann *et al.*, 2022). Winter soft wheat is a crucial crop in Ukraine and other countries with comparable climatic conditions. It is essential for the production of bread and other food products and is the main source of nutrition for millions of people. The problem of global warming and climate change (Sala & Herbei, 2023) may affect the cultivation of this crop. One of the major issues is the increase in winter temperatures, which can cause disruptions in plant development and a substantial correction in the timing of certain phases. As a result, yields may decrease.

To overcome these challenges, new genotypes of winter wheat are being developed based on either forms of different ecological and geographical origin or the creation of new forms from local material (Nazarenko *et al.*, 2021). The development of more efficient crop improvement methods could also be useful for ensuring sustainable winter wheat production under climate change (Jalal *et al.*, 2021). Managing climate change and adapting the agricultural sector to new conditions is a vital task for ensuring food security.

The purpose of this study was to identify the specific features of heritable variability of winter wheat varieties based on the frequency and range of positive changes arising from the interaction between the nature of the factor and the characteristics of the genotype.

## MATERIALS AND METHODS

The field experiments were conducted in the experimental fields of the Educational and Research Centre of Dnipro State Agrarian and Economic University (48°51'10"N, 35°25'31"E) in 2018-2022 (second to sixth generation of mutants). The experimental plots have a homogeneous cover consisting of ordinary low-humus, leached, medium loamy chernozem on loam. The experimental field is located in the Dnipro district of the Dnipro region of Ukraine, which belongs to the Northern subzone of the Steppe with insufficient moisture. Its climatic resources are characterised by the following indicators: hydrothermal coefficient >0.9, rare precipitation during the growing season – 250-280 mm, annual precipitation – 450-490 mm, and the sum of temperatures during the period with temperatures above 10°C – about 2,900°C.

Winter wheat seeds (1,000 grains per each concentration and water) of eight varieties: Balaton (Germany), Borovytsia, Zeleny Gai, Zoloto Ukrainy, Kalancha, Niva Odeska, Polyanka, Pochayna (Ukrainian selection) were treated with EMS (ethyl methane sulphonate) 0.025%, 0.05%, 0.1%; SA (sodium azide) 0.01%, 0.025%, 0.05%, 0.1%; DMS (dimethyl sulphate) 0.0125%, 0.025%, 0.05% (Sigma-Aldrich, Germany). The exposure to chemical supermutagen treatment was 24 hours following the generally accepted protocols. These concentrations are trivial for mutagens (chemical supermutagens) of this group. Controls were soaked in water.

In the second and third generations, possible mutations were examined visually and by manual reseeded by families (1-2-row plots, row spacing – 0.15 m, row spacing length – 1.5 m), and the inheritance of the newly identified traits was studied. The mutation frequency was calculated as the ratio of mutation cases to the total number of families investigated in the variant in percentage terms. Mutant lines were analysed to measure yield and quality in the fourth to sixth generation. The area of the plots was 5-10 m<sup>2</sup>, depending on the year of the experiment and generation, repetition was 1-2 times, standard (original variety and national variety Podolianka for determining productive forms) – every 20 numbers. The protein content of wheat grain was measured using an Infratec 1241 (FOSS, Denmark), and the content of glutenins and gliadins was measured by liquid chromatography using an RP-HPLS (INRA, France). The content of trace elements was determined using an Agilent 5110 inductively coupled plasma atomic emission spectrometer (Agilent, Germany) with light intensity based on wavelength characteristics. A multi-element test solution (Mg, Mn, Zn, Mo, Co, Cu) was used as Agilent standards.

Statistical analysis of data was performed according to ANOVA, the data was grouped and evaluated using discriminant and cluster analysis (Euclidean distance, single link) (Statistica 10.0, multivariate statistics module, TIBCO, Palo Alto, USA). The normality of the

data distribution was verified using the Shapiro-Wilk W-test. The differences between the individual variants were assessed using the Tukey test (pairwise comparison). The study of the plant material obtained by the treatment took place in two stages. During the first one, the second and third generations were used to identify changes in the original forms and to detect their inheritance by visual and biometric analysis. The resulting lines were again tested for heritability and their yield parameters were determined in a trial during the fourth to sixth generation. In parallel, small, hidden micromutations were analysed for biochemical components (protein, gluten, certain components of wheat grain reserve proteins significant for the baking industry, and the presence of some valuable trace elements). A total of 48,800 families were investigated for the three substances, of which 4,171 mutant lines were identified.

The following types of changes can be used as positive mutations: thick stem, short stem, semi-dwarfism, dwarfism, intensive wax coating (associated with high drought tolerance, in the variety model for the Northern Steppe), coarse grain, long ear, large ear, early maturity, disease tolerance (evaluation and selection for these traits started in generations M<sub>2</sub>-M<sub>3</sub>), productivity, ability to tillering (preliminary evaluation and selection in generations M<sub>2</sub>-M<sub>3</sub>, environmental testing in generations M<sub>4</sub>-M<sub>6</sub>), increased protein content, positive changes in protein components (content of high and low molecular weight glutenins and gliadins), positive changes in the content of trace elements (Mg, Mn, Zn, Mo, Co, Cu) (evaluation and selection in environmental testing of generations M<sub>4</sub>-M<sub>6</sub>).

Experimental studies of plants (both cultivated and wild), including the collection of plant material, were following the institutional, national, or international guidelines. The authors followed the standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1979).

## RESULTS AND DISCUSSION

Generally, all varieties belong to stable genotypes and the level of spontaneous variability is low. The results of the first mutagen, EMS, are presented in Table 1. A total of 15,800 families were investigated for controls and variants, including 1,043 mutant lines in total. The number of useful changes (possible complex changes, two or more per line) was 267, including negative aspects (additional changes) of 159 lines, of which 68 forms were finally tested as promising forms, which were of great interest for direct use but mainly as donors of traits (genetically valuable). The number of families in each treatment was 500, except for Balaton, EMS 0.1%, and Niva Odeska, EMS 0.1%, for other genotypes and concentrations, the depressant effect did not affect the amount of material for evaluation.

**Table 1.** Frequency of positive mutations in the fourth to sixth generation, EMS, ( $\bar{x} \pm SD$ ,  $n=400-500$ )

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.04 <sup>a</sup>	0.50	1	0	0.00±0.00 <sup>a</sup>
Balaton, EMS 0.025%	1.40±0.11 <sup>b</sup>	0.21	5	3	0.60±0.07 <sup>b</sup>
Balaton, EMS 0.05%	2.20±0.17 <sup>c</sup>	0.24	6	2	0.40±0.06 <sup>c</sup>
Balaton, EMS 0.1%	3.25 ±0.20 <sup>d</sup>	0.22	7	2	0.50±0.09 <sup>bc</sup>
Zoloto Ukrainy	0.40±0.08 <sup>a</sup>	0.33	1	0	0.00±0.00 <sup>a</sup>
Zoloto Ukrainy, EMS 0.025%	2.00±0.17 <sup>b</sup>	0.26	6	3	0.60±0.07 <sup>b</sup>
Zoloto Ukrainy, EMS 0.05%	3.40±0.22 <sup>c</sup>	0.31	11	4	0.80±0.09 <sup>b</sup>
Zoloto Ukrainy, EMS 0.1%	1.80±0.16 <sup>b</sup>	0.13	6	3	0.60±0.07 <sup>b</sup>
Zeleny Gai	0.20±0.04 <sup>a</sup>	0.33	1	0	0.00±0.00 <sup>a</sup>
Zeleny Gai, EMS 0.025%	2.40±0.18 <sup>b</sup>	0.44	7	3	0.60±0.06 <sup>b</sup>
Zeleny Gai, EMS 0.05%	2.60±0.19 <sup>b</sup>	0.36	6	3	0.60±0.06 <sup>b</sup>
Zeleny Gai, EMS 0.1%	2.00±0.17 <sup>b</sup>	0.21	5	2	0.40±0.04 <sup>c</sup>
Niva Odeska	0.00±0.00 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Niva Odeska, EMS 0.025%	1.80±0.13 <sup>b</sup>	0.26	7	3	0.60±0.08 <sup>b</sup>
Niva Odeska, EMS 0.05%	2.40±0.18 <sup>c</sup>	0.25	6	3	0.60±0.08 <sup>b</sup>
Niva Odeska, EMS 0.1%	3.50±0.29 <sup>d</sup>	0.22	8	3	0.75±0.11 <sup>b</sup>
Borovytsia	0.20±0.06 <sup>a</sup>	0.25	0	0	0.00±0.00 <sup>a</sup>
Borovytsia, EMS 0.025%	1.20±0.11 <sup>b</sup>	0.21	5	3	0.60±0.07 <sup>b</sup>
Borovytsia, EMS 0.05%	2.60±0.16 <sup>b</sup>	0.35	8	4	0.80±0.11 <sup>c</sup>
Borovytsia, EMS 0.1%	3.00±0.21 <sup>b</sup>	0.29	6	2	0.40±0.06 <sup>ab</sup>
Kalancha	0.40±0.01 <sup>a</sup>	0.40	1	0	0.00±0.00 <sup>a</sup>
Kalancha, EMS 0.025%	1.40±0.12 <sup>b</sup>	0.23	5	3	0.60±0.07 <sup>b</sup>
Kalancha, EMS 0.05%	2.80±0.19 <sup>c</sup>	0.34	9	4	0.80±0.09 <sup>b</sup>
Kalancha, EMS 0.1%	3.00±0.27 <sup>c</sup>	0.23	8	3	0.60±0.07 <sup>b</sup>
Polyanka	0.20±0.04 <sup>a</sup>	0.50	1	0	0.00±0.00 <sup>a</sup>
Polyanka, EMS 0.025%	1.60±0.10 <sup>b</sup>	0.35	6	3	0.60±0.07 <sup>b</sup>
Polyanka, EMS 0.05%	1.60±0.11 <sup>b</sup>	0.24	5	2	0.40±0.05 <sup>b</sup>
Polyanka, EMS 0.1%	2.20±0.16 <sup>c</sup>	0.25	6	3	0.60±0.07 <sup>b</sup>
Pochayna	0.20±0.04 <sup>a</sup>	0.50	1	0	0.00±0.00 <sup>a</sup>
Pochayna, EMS 0.025%	1.40±0.10 <sup>b</sup>	0.29	5	3	0.60±0.07 <sup>b</sup>
Pochayna, EMS 0.05%	1.60±0.11 <sup>b</sup>	0.25	5	2	0.40±0.05 <sup>b</sup>
Pochayna, EMS 0.1%	1.80±0.12 <sup>bc</sup>	0.20	5	2	0.40±0.05 <sup>b</sup>

**Note:** indicates a significant difference at  $P < 0.05$  for the Tukey's test with Bonferroni correction. Comparison within the same variety

**Source:** compiled by the authors

Only when compared to the standard is the number of beneficial changes in all varieties statistically significantly higher. Only in Balaton and Niva Odeska varieties a significant increase in the frequency of beneficial changes was observed with each increase in EMS concentration, in Zoloto Ukrainy the frequency first increased and then decreased at the third concentration to the level of the first one ( $F=2.26$ ;  $F_{0.05}=4.99$ ;  $P=0.08$ ). In Borovytsia and Zeleny Gai varieties, the variability under the influence of all concentrations was at the same level, in Kalancha there was no significant difference under the influence of the second and third concentrations ( $F=4.06$ ;  $F_{0.05}=4.99$ ;  $P=0.06$ ); for the Polyanka genotype – the first and second ( $F=3.22$ ;  $F_{0.05}=4.99$ ;  $P=0.07$ ); for Pochayna – the third differed from the first ( $F=7.11$ ;

$F_{0.05}=4.99$ ;  $P=0.02$ ), but did not differ from the second ( $F=4.01$ ;  $F_{0.05}=4.99$ ;  $P=0.06$ ), which did not have a significant difference between them ( $F=3.55$ ;  $F_{0.05}=4.99$ ;  $P=0.07$ ). As for the general characteristic of the parameter, the variability for the mutagen concentration was generally significant ( $F=17.23$ ,  $F_{0.05}=3.86$ ,  $P=0.001$ ), and the difference in the response for varieties was also significant ( $F=18.17$ ,  $F_{0.05}=4.11$ ,  $P=0.001$ ). As for the genotype-mutagenic interaction, it was significant only for Borovytsia and Niva Odeska ( $F=8.33$ ;  $F_{0.05}=3.10$ ;  $P=0.01$ ).

The proportion of useful changes in the total frequency reached 0.2-0.4 for different variants but was generally slightly higher for the first to third concentration. No statistically significant relationships were found. The same applies to the number of valuable

lines, except for the genotypes Kalancha, Polyanka, and Pochayna. The large number of additional negative changes and their complex nature led to a slight decrease in lines at the highest concentration, despite the higher frequency of useful forms in general (mainly semi-dwarfs and dwarfs).

The last parameter, the frequency of selected promising lines as a percentage of the total amount of material, is quite important. The indicator is much less variable than the frequency of valuable forms in general, but it can be said that half of the varieties (Zoloto Ukrainy, Borovytsia, Kalancha, Polyanka) have more or less the same level of the trait at any concentration, but a significant difference with the control. For Pochayna and Zeleny Gai, there was a slight gradual decrease, Niva Odeska showed an increase, while Balaton was unstable. In any case, the effect of different concentrations was significant ( $F=5.01$ ,  $F_{0.05}=3.86$ ,  $P=0.04$ ), the difference by genotype was not ( $F=3.98$ ,  $F_{0.05}=4.11$ ,  $P=0.06$ ), as was the genotype-mutagenic interaction ( $F=2.03$ ,  $F_{0.05}=3.10$ ,  $P=0.07$ ). Thus, it was difficult to find promising compositions among the available varieties

under the influence of EMS to create a system with an increased yield of useful forms.

Under the action of sodium azide, considering the greater variety of concentrations (which is mainly due to the wide range of agents in inducing changes and substantially less general knowledge of the ecogenetic factor), the largest amount of source material of 19,400 families in the second and third generations was investigated, of which 1,692 mutant lines were obtained and 301 lines were selected as potentially useful, 178 forms were proposed for further genetic improvement as donors of valuable traits and direct use (Table 2). The number of families in the variants for higher concentrations was reduced to 400, except for the more resistant Pochayna and Polyanka varieties. Thus, the negative effects had a greater impact on the viability of the samples than the previous factor. The same range of beneficial changes was observed for the mutagen, but the variability for most traits was substantially higher, primarily in the induction of shorter stemmed forms ( $F=21.17$ ;  $F_{0.05}=3.05$ ;  $P=1.94 \cdot 10^{-5}$ ) and positive biochemical changes ( $F=52.63$ ;  $F_{0.05}=3.01$ ;  $P=3.47 \cdot 10^{-8}$ ).

**Table 2.** Frequency of positive mutations in the fourth to sixth generation, SA, ( $\bar{x} \pm SD$ ,  $n=400-500$ )

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.03 <sup>a</sup>	0.50	0	0	0.00±0.00 <sup>a</sup>
Balaton, SA 0.01%	2.00±0.21 <sup>b</sup>	0.29	7	5	1.00±0.17 <sup>b</sup>
Balaton, SA 0.025%	3.20±0.25 <sup>c</sup>	0.33	11	7	1.40±0.25 <sup>b</sup>
Balaton, SA 0.05%	3.60±0.28 <sup>c</sup>	0.26	12	7	1.40±0.26 <sup>b</sup>
Balaton, SA 0.1%	3.50±0.28 <sup>c</sup>	0.17	6	3	0.75±0.12 <sup>bc</sup>
Zoloto Ukrainy	0.40±0.08 <sup>a</sup>	0.33	1	0	0.00±0.00 <sup>a</sup>
Zoloto Ukrainy, SA 0.01%	2.20±0.21 <sup>b</sup>	0.35	6	5	1.00±0.12 <sup>b</sup>
Zoloto Ukrainy, SA 0.025%	2.60±0.22 <sup>b</sup>	0.33	9	6	1.20±0.15 <sup>b</sup>
Zoloto Ukrainy, SA 0.05%	4.00±0.29 <sup>c</sup>	0.31	11	7	1.40±0.16 <sup>bc</sup>
Zoloto Ukrainy, SA 0.1%	4.25±0.34 <sup>c</sup>	0.22	12	5	1.25±0.15 <sup>b</sup>
Zeleny Gai	0.40±0.08 <sup>a</sup>	0.67	0	0	0.00±0.00 <sup>a</sup>
Zeleny Gai, SA 0.01%	1.80±0.17 <sup>b</sup>	0.33	5	4	0.80±0.11 <sup>b</sup>
Zeleny Gai, SA 0.025%	2.60±0.21 <sup>c</sup>	0.32	9	5	1.00±0.12 <sup>b</sup>
Zeleny Gai, SA 0.05%	3.00±0.25 <sup>c</sup>	0.26	9	5	1.00±0.12 <sup>b</sup>
Zeleny Gai, SA 0.1%	4.00±0.30 <sup>d</sup>	0.21	10	5	1.25±0.15 <sup>bc</sup>
Niva Odeska	0.00±0.11 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Niva Odeska, SA 0.01%	2.20±0.25 <sup>b</sup>	0.28	7	4	0.80±0.11 <sup>b</sup>
Niva Odeska, SA 0.025%	3.00±0.29 <sup>c</sup>	0.28	9	5	1.80±0.21 <sup>c</sup>
Niva Odeska, SA 0.05%	4.60±0.35 <sup>d</sup>	0.32	12	7	1.40±0.16 <sup>d</sup>
Niva Odeska, SA 0.1%	4.00±0.30 <sup>d</sup>	0.20	10	6	1.50±0.17 <sup>dc</sup>
Borovytsia	0.20±0.03 <sup>a</sup>	0.25	0	0	0.00±0.00 <sup>a</sup>
Borovytsia, SA 0.01%	2.20±0.21 <sup>b</sup>	0.38	7	4	0.80±0.11 <sup>b</sup>
Borovytsia, SA 0.025%	2.60±0.22 <sup>b</sup>	0.33	8	5	1.00±0.12 <sup>b</sup>
Borovytsia, SA 0.05%	3.60±0.31 <sup>c</sup>	0.30	11	6	1.20±0.14 <sup>bc</sup>
Borovytsia, SA 0.1%	3.50±0.30 <sup>c</sup>	0.18	9	5	1.25±0.14 <sup>bc</sup>
Kalancha	0.40±0.07 <sup>a</sup>	0.40	1	0	0.00±0.00 <sup>a</sup>
Kalancha, SA 0.01%	3.60±0.28 <sup>b</sup>	0.58	9	6	1.20±0.14 <sup>b</sup>

Table 2. Continued

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Kalancha, SA 0.025%	3.40±0.31 <sup>b</sup>	0.44	11	5	1.00±0.11 <sup>b</sup>
Kalancha, SA 0.05%	4.40±0.34 <sup>c</sup>	0.34	11	7	1.40±0.16 <sup>bc</sup>
Kalancha, SA 0.1%	5.50±0.36 <sup>d</sup>	0.26	14	8	2.00±0.19 <sup>d</sup>
Polyanka	0.00±0.00 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Polyanka, SA 0.01%	2.60±0.23 <sup>b</sup>	0.59	6	5	1.00±0.11 <sup>b</sup>
Polyanka, SA 0.025%	4.00±0.32 <sup>c</sup>	0.61	11	7	1.40±0.15 <sup>c</sup>
Polyanka, SA 0.05%	2.80±0.25 <sup>b</sup>	0.26	9	5	1.00±0.11 <sup>b</sup>
Polyanka, SA 0.1%	3.40±0.27 <sup>d</sup>	0.27	10	5	1.00±0.11 <sup>b</sup>
Pochayna	0.00±0.00 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Pochayna, SA 0.01%	2.00±0.21 <sup>b</sup>	0.43	6	5	1.00±0.11 <sup>b</sup>
Pochayna, SA 0.025%	3.00±0.26 <sup>c</sup>	0.44	11	6	1.20±0.14 <sup>b</sup>
Pochayna, SA 0.05%	3.20±0.27 <sup>d</sup>	0.31	11	6	1.20±0.14 <sup>b</sup>
Pochayna, SA 0.1%	4.00±0.32 <sup>e</sup>	0.33	10	7	1.40±0.16 <sup>bc</sup>

**Note:** indicates a significant difference at  $P < 0.05$  for the Tukey's test with Bonferroni correction. Comparison within the same variety

**Source:** compiled by the authors

The index of frequency of variability in the spectrum of useful changes shows that for all variants the difference with the control was statistically significant, and the variability by genotype ( $F=18.21$ ;  $F_{0.05}=3.55$ ;  $P=3.56 \cdot 10^{-4}$ ) and increasing concentration was significant ( $F=34.82$ ;  $F_{0.05}=3.11$ ;  $P=4.17 \cdot 10^{-7}$ ). The genotype-mutagen interaction was significant ( $F=24.17$ ;  $F_{0.05}=3.01$ ;  $P=5.52 \cdot 10^{-6}$ ). Only in Pochayna did the frequency increase statistically significantly with each concentration. In the varieties Zoloto Ukrainy ( $F=4.82$ ,  $F_{0.05}=5.17$ ,  $P=0.06$ ), Kalancha ( $F=4.44$ ,  $F_{0.05}=5.17$ ,  $P=0.07$ ), and Borovytsia ( $F=4.76$ ,  $F_{0.05}=5.17$ ,  $P=0.06$ ) there was no difference between the effect of the first and second concentrations. There was no difference between the second and third concentration in Balaton ( $F=3.11$ ,  $F_{0.05}=5.17$ ,  $P=0.09$ ) and Zeleny Gai ( $F=3.82$ ,  $F_{0.05}=5.17$ ,  $P=0.08$ ), in the Polyanka variety there was a difference ( $F=7.92$ ;  $F_{0.05}=5.17$ ;  $P=0.02$ ), but the frequency decreased before the first concentration ( $F=4.12$ ;  $F_{0.05}=5.17$ ;  $P=0.08$ ). In terms of the activity of the third and highest concentration, there was no difference in Balaton ( $F=4.82$ ;  $F_{0.05}=5.05$ ;  $P=0.07$ ), Zoloto Ukrainy ( $F=4.22$ ;  $F_{0.05}=5.05$ ;  $P=0.07$ ), Niva Odeska ( $F=3.81$ ;  $F_{0.05}=5.05$ ;  $P=0.08$ ), and Borovytsia ( $F=3.42$ ;  $F_{0.05}=5.05$ ;  $P=0.09$ ), while in all other varieties it increased significantly. Thus, in this case, the use of an SA concentration of 0.1% is justified for most cases.

In the total frequency, the proportion of valuable changes varied from 0.2 to 0.6, with a gradual decrease with increasing concentration for most genotypes upon reaching higher concentrations, with a possible gradual increase of SA within 0.01-0.05%. No significant trends were observed in the number of promising lines, except for the increase in complex changes at higher concentrations. According to the analysis of the frequency of selected lines in any variant, there was a difference

with the control, only in the varieties Niva Odeska and Kalancha there was some significant variability, which was expressed in a significant increase under the influence of higher concentrations. Generally, the frequency gradually increased moderately, or was not lower than the previous one, except for Balaton and Zoloto Ukrainy, where it decreased at 0.1% SA. For all varieties, the genotype-mutagen interaction was positive for Balaton ( $F=9.17$ ;  $F_{0.05}=4.22$ ;  $P=0.002$ ), Kalancha ( $F=9.98$ ;  $F_{0.05}=4.22$ ;  $P=0.001$ ), Polyanka ( $F=10.10$ ;  $F_{0.05}=4.22$ ;  $P=0.001$ ), and Pochayna varieties ( $F=13.16$ ;  $F_{0.05}=4.22$ ;  $P=4.13 \cdot 10^{-5}$ ). The effect of sodium azide as a mutagen differed significantly from EMS in that it has a much higher genetic activity both in terms of the frequency of individual changes in traits and the total number of genetically and breeding-value changes.

The effect of DMS led to a substantial decrease in the amount of plant material in the first generation, specifically, at a concentration of 0.05%, the number of families in some cases (Balaton, Niva Odeska) was reduced to 200. In total, 13,600 families in the second and third generation were studied as source material, from which 1,436 mutant lines were obtained (very high genetic activity of the factor influenced, and therefore with a substantial reduction in the amount of material, variability in some cases (mainly negative) exceeded the effect of SA, in some cases (mainly negative) exceeded the effect of SA and was much higher than the effect of EMS) and 226 lines were selected as potentially useful, 89 forms were proposed for further genetic improvement as donors of valuable traits and direct use (Table 2). The presence of numerous sterile forms and undesirable variability in ear shape had an impact. On the other hand, the ecogenetic factor was effective in mutations in stem structure and disease resistance and often induced rare types of mutations.

**Table 3.** Frequency of positive mutations in the fourth to sixth generation, DMS, ( $\bar{x} \pm SD$ ,  $n=200-500$ )

Variant	Frequency of positive changes, %	Share of total frequency	Number of lines, pcs.	Number of promising lines, pcs.	Frequency of promising lines, %
Balaton	0.20±0.05 <sup>a</sup>	0.50	0	0	0.00±0.00 <sup>a</sup>
Balaton, DMS 0.0125%	4.40±0.38 <sup>b</sup>	0.42	12	5	1.00±0.12 <sup>b</sup>
Balaton, DMS 0.025%	5.97±0.45 <sup>c</sup>	0.27	11	4	1.33±0.14 <sup>c</sup>
Balaton, DMS 0.05%	5.75±0.43 <sup>c</sup>	0.27	5	2	1.00±0.12 <sup>b</sup>
Zoloto Ukrainy	0.40±0.10 <sup>a</sup>	0.33	0	0	0.00±0.00 <sup>a</sup>
Zoloto Ukrainy, DMS 0.0125%	3.60±0.31 <sup>b</sup>	0.39	11	4	0.80±0.10 <sup>b</sup>
Zoloto Ukrainy, DMS 0.025%	4.50±0.39 <sup>c</sup>	0.23	12	3	1.00±0.12 <sup>b</sup>
Zoloto Ukrainy, DMS 0.05%	3.20±0.27 <sup>b</sup>	0.17	5	1	0.29±0.06 <sup>c</sup>
Zeleny Gai	0.40±0.10 <sup>a</sup>	0.67	0	0	0.00±0.00 <sup>a</sup>
Zeleny Gai, DMS 0.0125%	3.80±0.33 <sup>b</sup>	0.37	7	5	1.00±0.13 <sup>b</sup>
Zeleny Gai, DMS 0.025%	4.55±0.41 <sup>b</sup>	0.26	9	4	1.00±0.12 <sup>b</sup>
Zeleny Gai, DMS 0.05%	4.00±0.36 <sup>b</sup>	0.19	6	2	0.67±0.07 <sup>c</sup>
Niva Odeska	0.00±0.16 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Niva Odeska, DMS 0.0125%	4.40±0.40 <sup>b</sup>	0.43	11	4	0.80±0.21 <sup>b</sup>
Niva Odeska, DMS 0.025%	5.67±0.49 <sup>c</sup>	0.28	10	4	1.33±0.23 <sup>b</sup>
Niva Odeska, DMS 0.05%	4.00±0.43 <sup>b</sup>	0.23	9	3	1.50±0.24 <sup>bc</sup>
Borovytsia	0.60±0.08 <sup>a</sup>	0.75	1	1	0.20±0.09 <sup>a</sup>
Borovytsia, DMS 0.0125%	4.60±0.34 <sup>b</sup>	0.48	12	4	0.80±0.17 <sup>b</sup>
Borovytsia, DMS 0.025%	4.90±0.38 <sup>b</sup>	0.29	13	5	1.25±0.22 <sup>b</sup>
Borovytsia, DMS 0.05%	3.47±0.36 <sup>c</sup>	0.19	6	1	0.29±0.11 <sup>a</sup>
Kalancha	0.20±0.04 <sup>a</sup>	0.20	0	0	0.00±0.00 <sup>a</sup>
Kalancha, DMS 0.0125%	4.40±0.38 <sup>b</sup>	0.47	12	5	1.00±0.17 <sup>b</sup>
Kalancha, DMS 0.025%	6.60±0.43 <sup>c</sup>	0.31	13	6	1.50±0.21 <sup>b</sup>
Kalancha, DMS 0.05%	8.50±0.43 <sup>d</sup>	0.34	11	3	1.00±0.17 <sup>b</sup>
Polyanka	1.00±0.11 <sup>a</sup>	0.50	1	1	0.20±0.00 <sup>a</sup>
Polyanka, DMS 0.0125%	3.20±0.29 <sup>b</sup>	0.40	11	5	1.00±0.11 <sup>b</sup>
Polyanka, DMS 0.025%	3.97±0.31 <sup>c</sup>	0.31	11	6	1.33±0.14 <sup>b</sup>
Polanka, DMS 0.05%	4.60±0.40 <sup>c</sup>	0.26	9	3	0.75±0.09 <sup>bc</sup>
Pochayna	0.00±0.00 <sup>a</sup>	0.00	0	0	0.00±0.00 <sup>a</sup>
Pochayna, DMS 0.0125%	2.60±0.26 <sup>b</sup>	0.31	7	4	0.80±0.10 <sup>b</sup>
Pochayna, DMS 0.025%	3.13±0.30 <sup>b</sup>	0.24	6	3	0.60±0.08 <sup>b</sup>
Pochayna, DMS 0.05%	2.60±0.26 <sup>b</sup>	0.14	5	1	0.60±0.18 <sup>b</sup>

**Note:** indicates a significant difference at  $P < 0.05$  for the Tukey's test with Bonferroni correction. Comparison within the same variety

**Source:** compiled by the authors

In terms of the frequency of beneficial changes, it was statistically significantly different from the control at all concentrations. The variability of concentrations in the effect by variety was much lower, mainly due to the rapid increase in negative mutations at higher concentrations. Thus, Zeleny Gai and Pochayna varieties had no variability in concentration. Among the other varieties, the Borovytsia genotype ( $F=3.17$ ,  $F_{0.05}=4.99$ ,  $P=0.08$ ) had no significant difference between the first and second concentrations. In Balaton ( $F=5.06$ ;  $F_{0.05}=5.15$ ;  $P=0.06$ ) and Polyanka varieties ( $F=4.76$ ;  $F_{0.05}=5.15$ ;  $P=0.06$ ), there was no difference in mutagen activity under the influence of the second and

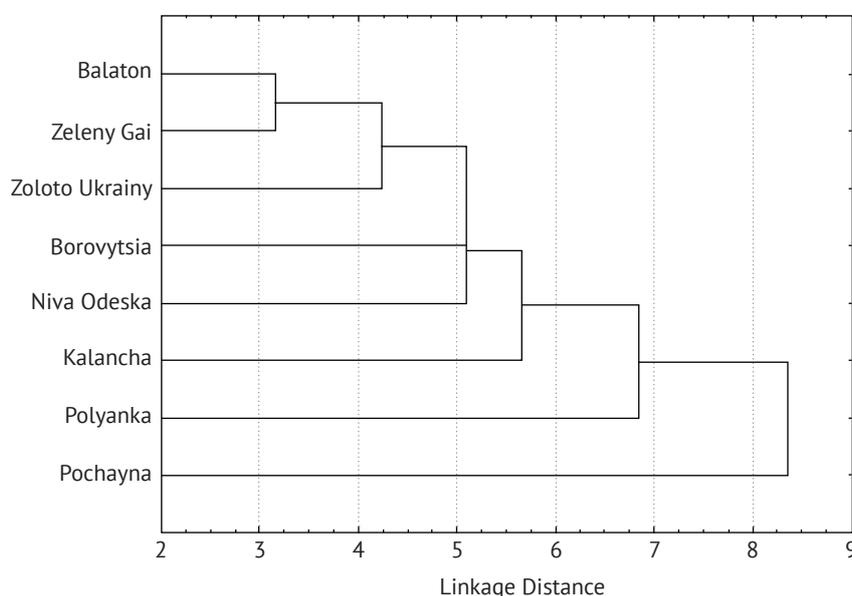
third concentrations; there was a difference in Zoloto Ukrainy ( $F=6.76$ ;  $F_{0.05}=5.15$ ;  $P=0.02$ ) and Niva Odeska ( $F=7.11$ ;  $F_{0.05}=5.15$ ;  $P=0.008$ ), but the frequency of beneficial changes falls to the level of the first concentration ( $F=4.16$ ;  $F_{0.05}=5.15$ ;  $P=0.07$  and  $F=4.92$ ;  $F_{0.05}=5.15$ ;  $P=0.06$ ). Again, only in the Kalancha variety did each subsequent concentration lead to a significant increase in the number of beneficial changes. The index of frequency of variability in the spectrum of useful changes showed that for all variants the difference with the control was statistically significant, and the variability according to genotype ( $F=19.77$ ;  $F_{0.05}=3.65$ ;  $P=2.26 \cdot 10^{-5}$ ) and increasing concentration was significant ( $F=14.82$ ;

$F_{0.05}=3.22$ ;  $P=5.09 \cdot 10^{-5}$ ). The genotype-mutagen interaction was also significant ( $F=5.17$ ;  $F_{0.05}=3.11$ ;  $P=0.02$ ).

The proportion of valuable changes in the total mutation frequency was 0.2-0.5, which was slightly lower than the SA, but generally quite high. No significant statistical patterns were found, except for a predominantly gradual decrease in the share of valuable changes in most varieties with increasing concentration and a relatively high level at 0.0125% DMS. Many additional negative changes resulted in a rather low number of forms selected for testing, which was significantly inferior to the effect of the previous mutagen and more comparable to EMS.

In terms of the frequency of such lines, it was found that this parameter is more variable than in the SA. Thus, there was no variability with the change in concentration

in the Niva Odeska, Kalancha, Polyanka, and Pochayna varieties, and it dropped to the level of the control under the influence of 0.05% DMS in Borovytsia. Balaton, Zoloto Ukrainy, and Zeleny Gai varieties had a gradual increase in the moderate range with a further decrease for higher concentrations. The genotype-mutagenic interaction was significant in a positive sense for the varieties Niva Odeska ( $F=7.08$ ,  $F_{0.05}=4.30$ ,  $P=0.007$ ), Kalancha ( $F=6.90$ ,  $F_{0.05}=4.30$ ,  $P=0.008$ ), and Polyanka ( $F=5.07$ ,  $F_{0.05}=4.30$ ,  $P=0.02$ ). According to the effect of different mutagens by nature, a cluster analysis was performed to consider the site-specific response of all genotypes depending on the group of substances and each substance separately (notably, there was no significant difference in the grouping by EMS and DMS, as further demonstrated by the discriminant analysis (Fig. 1).



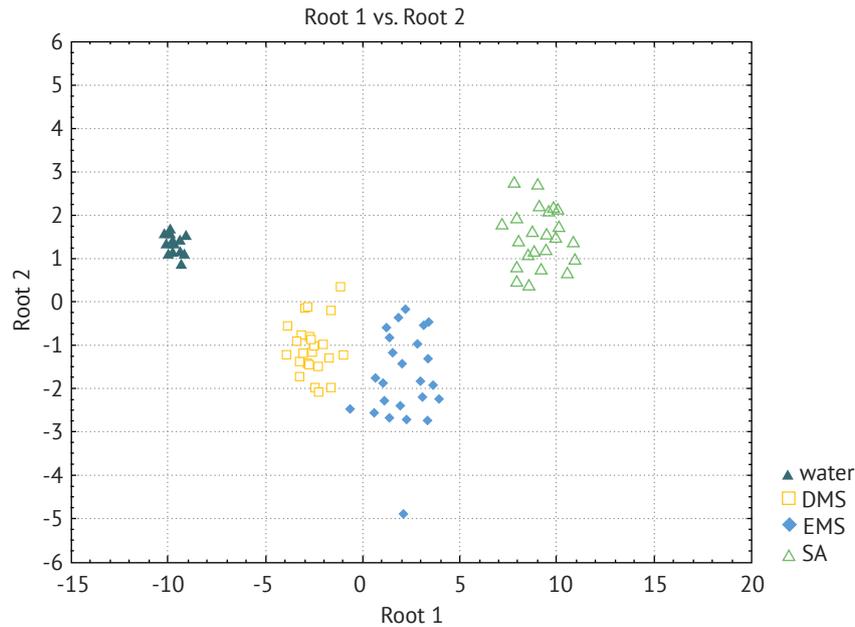
**Figure 1.** Results of cluster analysis by frequency of positive changes according to genotype

**Source:** compiled by the authors

In total, four groups were found, three of which were minor. The first group included Balaton, Zeleny Gai, Zoloto Ukrainy, Borovytsia, and Niva Odeska varieties, for which the genotype-mutagenic interaction was not factually significant despite the preliminary results of factor analysis for individual substances. The study found that the use of these genotypes to create a high-performance mutagen-initial form system is not possible for substances with high damaging properties. This does not mean that the use of this group will not lead to the emergence of promising forms, but the effectiveness of this process will be relatively mediocre. In the second group, one variety, Kalancha, showed a specific response to EMS and SA; due to the specific features of the genotype structure, it can be used as a component for enhancing mutational activity. The third group included the Polyanka variety, the fourth –

the Pochayna variety, which showed increased affinity for the action of SA. Considering the specific features of this factor, one should expect a relatively sizeable number of forms with biochemical and physiological microchanges.

The findings of the discriminant analysis performed according to mutagen (Fig. 2) suggested that EMS and DMS were actually more equally effective in terms of their effect on the entire group of genotypes. The effect of SA was the most variable and, clearly, SA as a mutagen has a more site-specific nature of action than other factors and is more dependent on the range of the used starting material on the particular subject of action. That is, systems with this factor are more likely to be selected and should be given further attention for the selection of new initial forms. A more thorough screening would also be warranted.



**Figure 2.** Identification of individual mutagenic factors according to genotype-mutagenic interaction

**Source:** compiled by the authors

In the spectrum of the obtained promising changes, the discriminant analysis identified signs that reliably show significant variability under the influence of certain substances (Table 4). An analysis was also carried out according to the individual characteristics of the mutation process. Thus, it was found that SA is more potent as a mutagen in terms of the range of induced traits (almost all traits except thick stem and yield), followed by DMS, which was not significantly effective in inducing mutations for intense waxy coat, large grain, large ear, high-yielding bushy forms, and higher protein content in wheat grain. The least successful in terms of the spectrum was the effect on the presented varietal set of EMS, which significantly affected the mutation frequencies for short stemmedness, induction of semi-dwarfs, forms with long ear, with positive changes in the content of trace elements. Analysing the traits, the

forms with a thick stem (resistant to lodging) should use only DMS, forms with intensive wax coating (potentially resistant to drought) and with large grain and ear – SA, bushy forms and those with a higher protein content – also SA. Thus, considering the higher efficiency of DMS in inducing dwarfs and disease-resistant forms, the use of SA and DMS is effective for this set of genotypes. The use of EMS is not advisable. Therewith, the emergence of yielding forms is not a model and cannot be predicted from the data obtained. Therefore, the data obtained need to be supplemented by expanding the variety of source forms.

Based on the results of a three-year small-plot environmental trial, the following mutant lines were obtained that were the most promising in terms of stable high yields (Table 5), and their characteristics in terms of grain quality are presented in Table 6.

**Table 4.** Identification of variability-significant features

Feature in the model	Wilks Lambda $\lambda$	Mutagen in the model	$F_{0.05}$	P-level
Thick stem	0.234	DMS	3.91	0.06
Short-stemmed	0.077	EMS, SA, DMS	18.12	0.001
Semi-dwarfs	0.089	EMS, SA, DMS	9.36	0.001
Dwarfs	0.157	SA, DMS	6.14	0.005
Intensive wax coating	0.212	SA	3.77	0.076
Large grain	0.213	SA	3.58	0.079
Long ear	0.084	EMS, SA, DMS	10.14	0.001
Large ear	0.286	SA	2.14	0.099
Early ripening	0.093	SA, DMS	8.04	0.004
Disease resistance	0.092	SA, DMS	7.77	0.003
Productive	0.229	absent	2.77	0.087

Table 4. Continued

Feature in the model	Wilks Lambda $\lambda$	Mutagen in the model	F <sub>0.05</sub>	P-level
Bushy	0.214	SA	4.80	0.046
Higher protein content	0.230	SA	4.43	0.039
Positive changes in protein components	0.104	SA, DMS	8.67	0.003
Positive changes in the content of trace elements	0.103	EMS, SA, DMS	8.98	0.003

Source: compiled by the authors

Table 5. Yield characteristics of the obtained mutant lines (Dnipro State Agrarian and Economic University, 2021-2023), t·ha<sup>-1</sup>

Variant	Origin	2021	2022	2023	Average
1	Podolianka, st.	5.38±0.09 <sup>a</sup>	5.56±0.08 <sup>a</sup>	6.22±0.12 <sup>a</sup>	5.72±0.10 <sup>a</sup>
26	Balaton, SA 0.025%	6.93±0.17 <sup>b</sup>	7.12±0.19 <sup>b</sup>	6.98±0.17 <sup>b</sup>	7.01±0.16 <sup>b</sup>
45	Zoloto Ukrainy, SA 0.05%	5.67±0.12 <sup>c</sup>	6.05±0.13 <sup>c</sup>	6.17±0.13 <sup>a</sup>	5.96±0.10 <sup>c</sup>
234	Zeleny Gai, EMS 0.05%	6.45±0.14 <sup>d</sup>	6.57±0.12 <sup>d</sup>	6.24±0.15 <sup>a</sup>	6.42±0.14 <sup>d</sup>
256	Niva Odeska, DMS 0.0125%	6.11±0.12 <sup>e</sup>	6.18±0.10 <sup>c</sup>	6.26±0.13 <sup>a</sup>	6.18±0.11 <sup>c</sup>
278	Borovytsia, EMS 0.025%	5.98±0.14 <sup>e</sup>	6.55±0.10 <sup>d</sup>	5.99±0.11 <sup>a</sup>	6.17±0.11 <sup>c</sup>
334	Kalancha, DMS 0.0125%	6.78±0.15 <sup>bd</sup>	6.99±0.11 <sup>b</sup>	6.34±0.12 <sup>a</sup>	6.70±0.12 <sup>bd</sup>
356	Kalancha, SA 0.05%	6.43±0.11 <sup>d</sup>	7.02±0.15 <sup>b</sup>	7.00±0.15 <sup>b</sup>	6.82±0.14 <sup>bd</sup>
391	Polyanka, SA 0.025%	6.24±0.11 <sup>ed</sup>	6.54±0.12 <sup>d</sup>	6.43±0.13 <sup>ac</sup>	6.40±0.12 <sup>d</sup>
412	Polyanka, DMS 0.0125%	6.79±0.14 <sup>bd</sup>	7.03±0.16 <sup>b</sup>	6.74±0.14 <sup>b</sup>	6.85±0.14 <sup>bd</sup>
423	Pochayna, SA 0.025%	7.01±0.15 <sup>b</sup>	7.12±0.16 <sup>b</sup>	7.07±0.14 <sup>b</sup>	7.07±0.15 <sup>b</sup>
446	Pochayna, SA 0.05%	6.77±0.10 <sup>bd</sup>	6.91±0.10 <sup>b</sup>	6.84±0.12 <sup>b</sup>	6.84±0.10 <sup>bd</sup>

Note: indicates a significant difference at  $P<0.05$  for the Tukey's test with Bonferroni correction. Comparison within the same variety

Source: compiled by the authors

Among the productive lines, all the original forms can be found in the origin, but the varieties Balaton, Zoloto Ukrainy, Zeleny Gai, Niva Odeska, and Borovytsia are represented by only one line each. The varieties Kalancha, Polyanka, and Pochayna each produced two lines. The use of SA in moderate concentrations yielded 6 lines, DMS – 3 lines, and EMS – 2 lines. The use of high concentrations that were at or above the LD<sub>50</sub> and RD<sub>50</sub> in the first generation did not produce any form for direct use (many genetically valuable forms, primarily dwarfs, semi-dwarfs, early maturing, and disease-resistant).

A total of 11 lines were identified for yielding qualities; the years of testing did not differ significantly in terms of soil and climatic conditions, but 2023 was the best year in terms of standard yields. Thus, in 2021, the productive lines were factually divided into 6 groups: the first was the Podolianka standard, the second line 45, which exceeded the standard but was inferior to other lines, the third lines 256 and 278, which exceeded the first and second groups but were inferior to others, the fourth line 234, 356, which exceeded the previous three groups, the fifth lines 26 and 423, which were record-breaking in 2021, and the intermediate position was occupied by the minor groups 334 and 412 (in terms of yield between the record-breaking and fourth

groups) and line 391 (in terms of yield between the third and fourth groups). Thus, lines 26, 334, 412, 423, mainly varieties with high genotype-mutagenic interaction, deserve more attention.

In 2022, the diversity was substantially lower, there were no transitional groups, and there was a clear differentiation into four groups, the first being the standard, the second lines 45, 256, which exceeded the standard but were inferior to other lines, the third 234, 278, 391, which exceeded the standard and the second group, and the fourth with the highest productivity lines 26, 356, 412, 423, 446. In 2023, the diversity was the lowest, with only three groups: the first standard and lines 45, 234, 256, 278, 334; the second transitional line 391, which did not differ from some lines in the first group but differed from others and the standard; and the third lines 26, 356, 412, 423, 446, which significantly exceeded the first and second groups. According to the results of three years, five groups were found, of which the cluster of lines 26, 423 ( $F=7.99$ ;  $F_{0.05}=3.89$ ;  $P=0.01$ ) was the most productive and the transitional 334, 356, 412, 446 ( $F=7.14$ ;  $F_{0.05}=3.71$ ;  $P=0.01$ ) were also interesting.

In terms of the technological qualities, 278 ( $F=5.09$ ;  $F_{0.05}=4.01$ ;  $P=0.03$ ), 234 ( $F=5.69$ ;  $F_{0.05}=4.01$ ;  $P=0.02$ ), 446

( $F=7.29$ ;  $F_{0.05}=4.01$ ;  $P=0.008$ ), 234 ( $F=5.00$ ;  $F_{0.05}=4.01$ ;  $P=0.03$ ), 391 ( $F=7.99$ ;  $F_{0.05}=5.98$ ;  $P=0.01$ ) were negatively affected, all the rest formed sufficient quality for

strong wheat. The traits are quite low in variation, unlike the following (content of reserve protein components) (Table 6).

**Table 6.** Technological qualities of wheat grain (Dnipro State Agrarian and Economic University, 2022)

Variety/line	Protein, %	Gluten, %	Glutenins		Gliadins
			HMW	LMW	
Podolianka	13.98 <sup>a</sup>	25.18 <sup>a</sup>	0.15999 <sup>a</sup>	0.46346 <sup>a</sup>	0.4574 <sup>a</sup>
26	14.07 <sup>a</sup>	25.99 <sup>a</sup>	0.17423 <sup>b</sup>	0.48435 <sup>b</sup>	0.4545 <sup>a</sup>
45	14.02 <sup>a</sup>	26.03 <sup>a</sup>	0.15245 <sup>a</sup>	0.48477 <sup>b</sup>	0.4553 <sup>a</sup>
234	13.51 <sup>b</sup>	22.19 <sup>b</sup>	0.16313 <sup>a</sup>	0.46569 <sup>a</sup>	0.4435 <sup>b</sup>
256	13.78 <sup>ab</sup>	24.56 <sup>a</sup>	0.15356 <sup>a</sup>	0.46223 <sup>a</sup>	0.4101 <sup>c</sup>
278	14.27 <sup>c</sup>	27.74 <sup>c</sup>	0.16405 <sup>a</sup>	0.42100 <sup>c</sup>	0.4124 <sup>c</sup>
334	14.27 <sup>c</sup>	26.97 <sup>ac</sup>	0.17341 <sup>b</sup>	0.45404 <sup>a</sup>	0.4557 <sup>a</sup>
356	14.01 <sup>a</sup>	25.78 <sup>a</sup>	0.18513 <sup>b</sup>	0.45313 <sup>a</sup>	0.4416 <sup>b</sup>
391	13.53 <sup>b</sup>	22.23 <sup>b</sup>	0.18022 <sup>b</sup>	0.40719 <sup>d</sup>	0.4512 <sup>a</sup>
412	14.02 <sup>a</sup>	26.10 <sup>a</sup>	0.19403 <sup>c</sup>	0.46300 <sup>a</sup>	0.4548 <sup>a</sup>
423	14.15 <sup>ac</sup>	26.78 <sup>c</sup>	0.18478 <sup>b</sup>	0.41999 <sup>d</sup>	0.4618 <sup>a</sup>
446	14.52 <sup>d</sup>	28.17 <sup>d</sup>	0.19357 <sup>c</sup>	0.40976 <sup>d</sup>	0.4639 <sup>ad</sup>

**Note:** indicates a significant difference at  $P<0.05$  for the Tukey's test with Bonferroni correction. Comparison within the same variety

**Source:** compiled by the authors

For durum wheat, a high content of high-molecular weight glutenins, a lower content of low molecular weight glutenins, and a high content of gliadins are desirable. According to the first variant, lines 26, 334, 356, 391, 412, 423, 446 had the best results. The low-molecular-weight glutenin content was lower in mutant forms 278, 391, 423, 446, and negative in 26 and 45. The gliadin content was higher only in line 446, and lower negative in lines 234, 256, 278, and 356. This combines high productivity and the best quality of lines 26 (negative for high low molecular weight glutenin), 423, 334, 356, 412, and separately, form 446 (combining high yield and best quality in all parameters).

Thus, for the material studied, it is advisable to use mainly SA in concentrations of 0.025-0.05% and DMS in concentrations of 0.0125% in the complex as mutagens, and while the effectiveness of SA has already been discussed, DMS is substantially understudied. EMS as a mutagen is limited in its effectiveness both in terms of characteristics and material yield. It is possible to obtain forms with a high damaging effect under the influence of moderate concentrations of mutagens that combine improvements in a complex of economically valuable traits. Based on the eight varieties studied and three mutagens at 10 concentrations, six systems with increased yields of valuable forms and an increase in efficiency of up to half of the baseline have already been identified, which indicates the prospects of searching in this area.

The analysis of the data obtained so far (M. Nazarenko *et al.*, 2022) showed a decrease in site

specificity with an increase in the activity and damage capacity of the mutagen. However, when using DMS and SA (as opposed to EMS), the trend was quite different (OlaOlorun *et al.*, 2021), and the response of a particular source material to the action was significant, as the pairwise comparison of genotypes suggests, which can be partially reproduced in the number of traits with a significant effect of a single factor, indicating the contrast of individual genotypes (Kalanča, Pochayna, Polyanka) and the possibility of creating high-performance systems based on them. The described regularity of the mutation process allows using this type of variability to obtain new material with the required potential in a more manageable, reliable, and predictable manner, which was previously considered impossible. Mutations in research have proven to be a reliable and constant source for local genetic resources, as indicated by previous studies by A. Anter (2021), for traits such as long grained ear, early maturity, with a set of valuable biochemical changes and disease resistance.

Previously obtained data by M. Hussain *et al.* (2021) show that the use of SA and DMS as mutagenic factors is advisable primarily to obtain forms for use as components (Mangi *et al.*, 2021) for further improvement of existing forms through recombinant breeding. It is less likely to create forms that can be used directly as commercial varieties in the future. This variant again stays outside the forecast (Ergün *et al.*, 2023a), despite a fairly large sample size. To increase the reliability of the forecast, as previously pointed out by M. Hassine *et al.* (2023), it is necessary to expand the set of genotypes used in the

study substantially. First of all, SA and DMS (additionally as mutagenic factors show a more significant dependence on the specific features of genotype-mutagenic interaction, i.e., they depend on the DNA structure of the source material of the site variety, as was mentioned earlier by H. Shimelis *et al.* (2019), with a clear differentiation from genetically determined responsiveness to mutagenic effects.

The regularities established during the study also demonstrate that a fairly significant number of traits that are definitely economically valuable are highly variable under the influence of the factors under study (Ergün *et al.*, 2023b) (with the exception of productive mutations). This once again indicates that in the case of chemical supermutagens, a great deal depends on a well-chosen starting material, depending on the particular factor. As the concentration of mutagenic substances increases, the risk of negative and positive changes in the complex continues to increase substantially (Shabani *et al.*, 2022). The number of such cases is quite considerable even for moderate concentrations, which is why up to half of all promising mutant forms obtained are ignored. This is a general trend (le Roux *et al.*, 2021). Varieties that demonstrate high variability both in the first generation in terms of the effects of mutagenic depression and in the second or third generation in terms of the frequency and spectrum of heritable changes are not always more suitable for use, but this trend is not absolute. In our research, two of the three promising varieties, Polyanka and Pochayna, are less vulnerable.

A close relationship was found between economically valuable traits and the frequency of valuable mutations obtained for these genotypes, the overall frequency and spectra of visually identifiable mutations. Although this pattern has already been identified (Mamenko & Yakymchuk, 2019), it was not for chemical mutagenesis. Thus, for the genetic improvement of winter wheat varieties, the six identified combinations of three varieties of Ukrainian breeding and moderate concentrations of sodium azide and dimethyl sulphate can be used, which guarantee a substantial increase in the yield of forms with economically valuable traits by about 20-50%.

## CONCLUSIONS

The genetic material of Ukrainian breeding (varieties Kalancha, Pochayna, Polyanka) is a successful component as an initial form for the creation, together with

the action of chemical supermutagens of high damage capacity (SA and DMS), of systems with a prominent stable yield of mutants with positive changes in economically valuable traits. That is, six systems based on three varieties and two mutagens (SA and DMS) were identified, the use of which led to guaranteed production of lines with positive complex shifts in valuable biochemical components, increased yield of useful forms (up to 50% compared to other variants). The forms obtained by applying the proposed systems can have additional valuable qualities, but the effectiveness of the variety-mutagen complex begins to decline with increasing concentration, primarily due to the increased risk of additional negative qualities. When obtaining such systems, it is justified to invest in early continuous screening of forms by biometric and biochemical analysis in the second or third generation after mutagenic exposure, which guarantees the availability of additional valuable material for genetic improvement of the crop. Thus, during the genetic improvement, it is worth using a moderate amount of sodium azide (0.025-0.05%) and, as an additional source of variability, moderate concentrations of DMS (0.0125%), while the use of EMS is less justified. Based on the results of research on the identification of beneficial changes and their correlations in the system of interaction between nature, factor concentrations, and characteristics of the source material, promising complexes of these components were identified, the combination of which leads to a substantial increase in the efficiency of the genetic improvement of winter wheat. Further research will focus on such valuable traits as drought and winter hardiness, specific features of mineral use and accumulation by the studied lines to confirm the parameters that provide advantages in yield and grain quality, and it is also planned to investigate the induction of new forms by highly active mutagens based on new Ukrainian and international forms with the same traits.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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## Спадкова мінливість у пшениці озимої при взаємодії генотипа з чинниками високої генетичної активності

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**Анотація.** Виявлення нових можливостей в застосуванні окремих екогенетичних чинників для генетичного поліпшення пшениці озимої є важливим компонентом вдосконалення існуючих агроценозів зернових культур в рамках стратегії продовольчої безпеки. Метою дослідження було показати можливості речовин з високою генетичною активністю в індукції потенційно-корисних змін та встановити можливість отримання оптимізованих схем для отримання нових форм та більш керованої мінливості за ознаками. Мутації виявляли візуальним спостереженням та біометричним, біохімічним аналізом починаючи з другого покоління до шостого с перевіркою успадкування виявлених ознак у наступних поколіннях. Показано можливості мутаційного процесу за окремими генетично- та селекційно-цінними формами, особливості індукції таких ознак, що дає змогу підвищити прогнозованість отримання необхідних мутантних ліній та частково-керованої високої варіативності за окремими ознаками. Встановлено, що найбільш перспективним для використання є комплексне застосування помірних доз азиду натрію та диметилсульфату як екогенетичних чинників. Показано, що в комплексі з використанням трьох сортів серед досліджуваних (Каланча, Полянка, Почайна) можливе створення вискоелективних генотип-мутагенних систем з підвищеним виходом цінних форм. Зростання мутагенної депресії при використанні більш шкочинних речовин може бути суттєво скомпенсованим підвищенням частки корисних змін. Негативним є їх комплексний характер дії. Природа екогенетичного фактору як чинник не менш суттєва ніж концентрація мутагену або генотип вихідної форми, що іноді ураховується недостатньо. Підтверджено, що використання наведених факторів є доволі обіцяючим з огляду отримання малих біохімічних змін (мікромутацій). Виділено декілька перспективних мутантних ліній, що мають комплексне поліпшення врожайності та якості зерна, без додаткових негативних змін. Результати дослідження можна використати в селекційних програмах для створення нових сортів пшениці озимої

**Ключові слова:** мутації; хімічний мутагенез; пшениця озима; супермутагени; врожайність; якість зерна

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