



## Original researches

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## Induction of positive changes for winter wheat under the action of a group of ecogenetic factors with lower damaging ability

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**Abstract.** The study of new agents for induction of practical biodiversity in local varieties of winter wheat for breeding process is a promising area in terms of obtaining both new commercial varieties and components for recombination breeding. The purpose of the study was to demonstrate the possibilities of low-damaging mutagens in terms of optimizing the yield and grain quality parameters of mutant forms. Winter wheat seeds of eight varieties (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Nyva Odeska, Polianka, Pochaina) were subjected by DAB (1,4-bisdiazoacetylbutane) 0.1%, 0.1%, 0.3%; NEU (nitrosoethylurea) 0.010%, 0.025%; NMU (nitrosomethylurea) 0.0125%, 0.025% (Sigma-Aldrich, Germany). The seeds were treated with 24 h exposure according to the generally recommended method for chemical mutagens actions. The regularity of the mutational process was monitored by agriculturally valuable traits, which makes the process of using experimental mutagenesis in order to obtain new forms with the required parameters more reliable and predictable. Studies have demonstrated that three varieties Kalancha, Polianka, Pochaina are promising in terms of variability of agriculturally valuable mutations to optimize the mutation process. Polianka and Pochaina were tested for NEU and NMU, Kalancha for all. The study revealed that NEU and NMU were more perspective in action for wheat genetic improvement, especially for biochemical changes, than DAB, which action is suitable for changes in size of spike. In general, traits for improvement were thick stem semi-dwarf, dwarf, intense epicuticular wax accumulation, large-size grain forms cannot be proper for low-damage ability mutagens, influence of which is effective for short-stem, early ripeness, and also tillering capacity, higher protein content, positive changes at protein components, and positive changes in microelement content. Only NMU can be used for mutations for tolerance of diseases, and NEU for changes in protein content as a regular mutation process. The possibility of producing forms with complex favorable changes has been confirmed, although their number is small. With increasing concentration, the likelihood of additional unfavorable traits, such as high stem or late ripeness, also increases in promising lines. Several new mutation lines of winter wheat were proposed as future commercial varieties with high grain productivity and sufficient bread-making qualities. One of these forms has excellent grain protein qualities. It is planned to analyze physiological changes in the produced lines, primarily in terms of resistance to abiotic factors such as drought tolerance and winter hardiness.

**Keywords:** genotype; 1,4-bisdiazoacetylbutane; nitrosomethylurea; nitrosoethylurea; mutations; ecogenetic.

### Introduction

The action of chemical mutagenesis on cultivated plants usually results in hereditary changes, which can be beneficial for genetic improvement in mutation breeding. Even a little single action significantly changes the development and heredity of crop such as winter wheat (Dwinanda et al., 2020).

Chemical supermutagens with low damaging ability are some of the most popular factors used not only for genetic improvement but also for studies of action of mutagens on plant organisms in general (starting from the model-plant genetic objects). Large number of successful valuable forms of the main cultivated crop is due to the action of chemical mutagenesis, which, taking into account the level of relevant biological studies, is broadly used for crossbreeding improvement through parent forms and to research gen systems for certain valuable traits and producing fundamentally new biochemical compositional traits in complex with other positive changes (Ariraman et al., 2018). To sum it up, in the XXI century, 189 principally new varieties were created by chemical mutagens: 53 in China, 17 in the USA, 9 in Russia and Iraq, 8 in India, 7 in Bangladesh and Pakistan, 2 in Vietnam, 1 in Poland, and 5 in Japan and Korea. Total of 65.2% of these forms were produced in Asia in the framework of the FAO/IAEA programs, and 12.0% in the European Union (Shimelis et al., 2019).

Several researchers believe that it is better to use chemical mutagens in high concentrations (due to more complex changes in several key traits). The available global statistics (Kumar et al., 2018) for some directions of genetic improvement supports this statement. Genetic improvement using average concentrations of chemical mutagens has been successful in creating productive mutant lines, resistant to disease and abiotic stresses. Action of chemical mutagens quite often leads to deep morphological changes in plants. Mutant forms with new phenotypes should have beneficial changes in the content of valuable biochemical substances and their components (Anter et al., 2021). The effect of a chemical mutagen is characterized by pronounced mutations, high number of morphoses and genocopies. Despite the fact that it is not the best choice for the highest mutational rate, chemical action on seeds is more effective in terms of subsequent mutational material. Increase in the general rate and spectrum in the cases of other subjects is not so effective due to further problems (Shabani et al., 2022).

Studies of the experimental mutagenesis of cereal crops in South-East Asia, according to the international scientific cooperation programs, have resulted in over 30 new varieties of crops, produced using chemical mutagens. This type of action is used more frequently and more efficiently (Bezie et al., 2020).

Chemical mutagens are of interest primarily because of their higher interaction with a specific genotype, site-specificity to native, original

DNA at high level, which is less normal for physical agents (Jalal et al., 2021). Therefore, several extremely promising opportunities open up at once for the genetic improvement of agricultural crops, which are less typical when using other factors of variability (Abdel-Hamed et al., 2021).

Winter wheat is an extremely valuable grain food crop, especially for areas of unstable climate conditions, including the main farming part of Ukraine (Nazarenko et al., 2021). Problems with the global warming and climate change lead to the advancement of more heat-loving crops to the north, mitigation of overwintering conditions, which is especially important for winter crops (Hongjie et al., 2019; Harkness et al., 2020).

The main objective of our study was to show the peculiarities of the hereditary variability of the local and non-local winter-wheat varieties based on the rate and spectrum of positive changes (including mutations by biochemical traits, the possibilities of chemical agents with low damaging ability for improvement through genotype-mutagen interaction, site-specificity for genetic improvement in wide and narrow sense.

## Materials and methods

The research was carried out in the experimental fields of the Educational and Scientific Center of the Dnipro State Agrarian and Economic University (48°51'10" N, 35°25'31" E) in 2021–2023 (M4–M6). The experimental areas have a homogeneous cover, consisting of ordinary low-humus, leached, medium-loamy black soil on a loamy soil. The content of nitrogen (according to Tiurin) during the years of research has not exceeded 3–5 mg, mobile phosphorus (according to Chyrykov) – 20–30 mg, exchangeable potassium (according to Chyrykov) – 20–35 mg per 100 g of dry soil.

The experimental field is located in the Dniprovskiy district of the Dnipropetrovsk Oblast, Ukraine, which is the northern warm and insufficiently wet area. Its climatic resources are characterized by the following indices: hydrothermal coefficient is >0.9, precipitation rare during the growing season – 250–280 mm, the annual amount of precipitation – 450–490 mm, total temperature for the period with temperatures above 10 °C – about 2,900 °C.

**Table 1**

Rate of positive changes in the forth – fifth generations, DAB ( $x \pm SD$ ,  $n = 500$ )

Variant	Rate of positive changes, %	Part from general rate, %	Number of lines	Number of promising lines	Rate of promising lines, %
Balaton	0.20 ± 0.04 <sup>a</sup>	0.50	1	0	0.00 ± 0.00 <sup>a</sup>
Balaton, DAB 0.1%	2.00 ± 0.18 <sup>b</sup>	0.43	10	2	0.40 ± 0.11 <sup>a</sup>
Balaton, DAB 0.2%	2.40 ± 0.19 <sup>b</sup>	0.38	11	4	0.80 ± 0.17 <sup>b</sup>
Balaton, DAB 0.3%	3.40 ± 0.21 <sup>c</sup>	0.41	14	6	1.20 ± 0.24 <sup>c</sup>
Zoloto Ukrainy	0.60 ± 0.10 <sup>a</sup>	0.75	2	0	0.00 ± 0.00 <sup>a</sup>
Zoloto Ukrainy, DAB 0.1%	2.20 ± 0.17 <sup>b</sup>	0.52	10	2	0.40 ± 0.04 <sup>b</sup>
Zoloto Ukrainy, DAB 0.2%	2.20 ± 0.18 <sup>b</sup>	0.39	9	4	0.80 ± 0.04 <sup>c</sup>
Zoloto Ukrainy, DAB 0.3%	3.00 ± 0.22 <sup>c</sup>	0.41	12	6	1.20 ± 0.23 <sup>d</sup>
Zelenyi Hai	0.40 ± 0.10 <sup>a</sup>	0.67	2	0	0.00 ± 0.00 <sup>a</sup>
Zelenyi Hai, DAB 0.1%	1.80 ± 0.15 <sup>b</sup>	0.41	8	3	0.60 ± 0.17 <sup>b</sup>
Zelenyi Hai, DAB 0.2%	2.40 ± 0.18 <sup>c</sup>	0.40	11	5	1.00 ± 0.21 <sup>c</sup>
Zelenyi Hai, DAB 0.3%	3.00 ± 0.21 <sup>d</sup>	0.38	13	5	1.00 ± 0.22 <sup>c</sup>
Niva Odeska	0.60 ± 0.21 <sup>a</sup>	0.50	3	0	0.00 ± 0.00 <sup>a</sup>
Nyva Odeska, DAB 0.1%	2.20 ± 0.14 <sup>b</sup>	0.65	11	1	0.20 ± 0.09 <sup>a</sup>
Nyva Odeska, DAB 0.2%	2.80 ± 0.19 <sup>c</sup>	0.56	12	6	1.20 ± 0.24 <sup>b</sup>
Nyva Odeska, DAB 0.3%	3.40 ± 0.23 <sup>d</sup>	0.49	14	5	1.00 ± 0.22 <sup>b</sup>
Borovytsia	0.40 ± 0.08 <sup>a</sup>	0.40	2	0	0.00 ± 0.00 <sup>a</sup>
Borovytsia, DAB 0.1%	1.80 ± 0.14 <sup>b</sup>	0.45	8	2	0.40 ± 0.14 <sup>b</sup>
Borovytsia, DAB 0.2%	2.60 ± 0.18 <sup>c</sup>	0.45	12	4	0.80 ± 0.18 <sup>c</sup>
Borovytsia, DAB 0.3%	2.80 ± 0.18 <sup>c</sup>	0.40	11	4	0.80 ± 0.18 <sup>c</sup>
Kalancha	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Kalancha, DAB 0.1%	2.20 ± 0.18 <sup>b</sup>	0.44	11	2	0.40 ± 0.14 <sup>b</sup>
Kalancha, DAB 0.2%	3.80 ± 0.23 <sup>c</sup>	0.49	16	4	0.80 ± 0.19 <sup>c</sup>
Kalancha, DAB 0.3%	5.40 ± 1.29 <sup>d</sup>	0.47	23	5	1.00 ± 0.24 <sup>c</sup>
Polianka	0.20 ± 0.07 <sup>a</sup>	0.50	1	0	0.00 ± 0.00 <sup>a</sup>
Polianka, DAB 0.1%	2.60 ± 0.13 <sup>b</sup>	0.72	12	2	0.40 ± 0.11 <sup>b</sup>
Polianka, DAB 0.2%	4.20 ± 0.25 <sup>c</sup>	0.84	19	4	0.80 ± 0.19 <sup>c</sup>
Polianka, DAB 0.3%	3.40 ± 0.22 <sup>d</sup>	0.50	15	5	1.00 ± 0.24 <sup>c</sup>
Pochaina	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Pochaina, DAB 0.1%	2.60 ± 0.16 <sup>b</sup>	0.68	12	2	0.40 ± 0.12 <sup>b</sup>
Pochaina, DAB 0.2%	3.20 ± 0.20 <sup>c</sup>	0.62	14	3	0.60 ± 0.14 <sup>b</sup>
Pochaina, DAB 0.3%	3.40 ± 0.21 <sup>c</sup>	0.52	15	3	0.60 ± 0.14 <sup>b</sup>

Note: significant differences at  $P < 0.05$  according to the Tukey HSD test with Bonferroni's Correction; comparison in terms of one variety at columns; DAB – 1,4-bisdiazaoacetylbutane, NEU – nitrosomethylurea, NMU – nitrosomethylurea.

Seeds of winter wheat (1000 grains for each variant) were subjected to DAB (1,4-bisdiazaoacetylbutane) 0.1%, 0.1%, 0.3%; NEU (nitrosoethylurea) 0.010%, 0.025%; and NMU (nitrosomethylurea) 0.0125%, 0.025% (Sigma-Aldrich, Germany). The exposure lasted for 24 hours according to the generally recommended protocols for chemical mutagenesis (Spencer-Lopes et al., 2018; Nazarenko, 2021).

In the second and third generations, we studied the mutations visually and by yield of sown families (1–3-row plots, row spacing of 0.15 m, row length of 1.50 m), and carried out inheritance studies. The mutation rate was calculated as a ratio of mutant cases to the total number of families in percents. The mutant lines were analyzed to measure yield and quality parameters in the fourth-sixth generations. The area of the plots was 5–10 m<sup>2</sup>, depending on the year of experiment, the repetition was 1–2 times, and the control plots were after every 20 experimental plots. The protein content in wheat grain was measured on the Spektra RT device, the content of glutenins and gliadins by liquid chromatography on the RP-HPLS device. Development of the contents of microelements was monitored using Agilent 5110 inductively coupled plasma atomic emission spectrometer by the parameters of waves. A multi-element production solution was used as standards (Mg, Mn, Zn, Mo, Co, Cu).

Statistic analysis of the data was performed using ANOVA, grouping and estimation of the data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). Pairwise comparison was performed using the Tukey HSD test.

## Results

The study of the obtained plant material began with identification of the rate of positive changes in the second-third generations of the resulted mutant population and changes in yield and different parameters of quality in the next generations. All obtained lines were subsequently subcultivated to confirm the inheritance of their altered traits as mutations. In total, for water and families (lines) after mutagen action, 16,000 families in the second-third generation were examined, and 738 mutant lines were selected for DAB action (Table 1).

Number of valuable forms was 314. Total of 89 lines with positive changes without any negative additional traits were finally selected for the breeding process. The concentrations of mutation factor were important for mutation breeding process of winter wheat. The number of families was 500 for all variants.

As mutations, we considered thick stem, short-stem, semi-dwarf, dwarf, intense epicuticular wax accumulation, large-size grain, long spike, large-size spike, early ripeness, tolerance for diseases (evaluation and selection in  $M_2$ – $M_3$  generations), productivity, tillering capacity (preliminary evaluation and selection in  $M_2$ – $M_3$  generations, study by ecological examination in  $M_4$ – $M_6$  generations), higher protein content, positive changes in protein components (content of high and low-molecular-weight glutenins and gliadins), positive changes in microelement content (Mg, Mn, Zn, Mo, Co, Cu) (evaluation and selection in  $M_4$ – $M_6$  generations). In general, all the varieties belong to stable genotypes and the level of spontaneous variability is low. Moreover, as for modern varieties, with significantly lower genome stability, it is even lower.

As can be seen, the rate of positive changes was higher with more amount of mutagen for varieties Zelenyi Hai, Niva Odeska, Kalancha, and Polianka in all variants. For varieties Balaton ( $F = 3.17$ ;  $F_{0.05} = 4.99$ ;  $P = 0.07$ ), Zoloto Ukrainy ( $F = 3.22$ ;  $F_{0.05} = 4.99$ ;  $P = 0.07$ ), there were no differences between the first and second concentrations. For varieties Borovytsia ( $F = 2.32$ ;  $F_{0.05} = 4.99$ ;  $P = 0.08$ ) and Pochaina ( $F = 2.11$ ;  $F_{0.05} = 4.99$ ;  $P = 0.08$ ), there were differences in cases of DAB 0.2% and 0.3%. Variability of positive changes in all genotypes (up to 2.8–3.4%, without significant difference) were on the same level, with one exception for Kalancha (up to 5.4%) ( $F = 7.62$ ;  $F_{0.05} = 4.99$ ;  $P = 0.03$ ). Therefore, by this parameter, variety Kalancha demonstrated a gradual increase in valuable forms, but without additional negative changes in the form, the rate of which increased with increasing concentration in general. Induction of the overall rate of positive changes was affected by increase in the concentrations of DAB ( $F = 34.11$ ;  $F_{0.05} = 3.86$ ;  $P = 3.09 \cdot 10^{-3}$ ), but regarding differences in the initial variety ( $F = 3.01$ ;  $F_{0.05} = 4.11$ ;  $P = 0.07$ ). The genotype-mutagen interaction was significant only for variety Kalancha ( $F = 8.77$ ;  $F_{0.05} = 3.55$ ;  $P = 0.03$ ).

The share of the general rate was not high, about 0.4–0.6% for the most variants, except Polyanka. Another key parameter for mutation

breeding was the rate of promising lines (from general number of families, 500 in all cases) which was not higher with more quantity of mutagen for all varieties (less variable indicator), but rate of promising lines was influenced by increase in the concentrations of DAB ( $F = 11.10$ ;  $F_{0.05} = 3.86$ ;  $P = 0.007$ ) and no differences by the genotypes ( $F = 2.17$ ;  $F_{0.05} = 4.11$ ;  $P = 0.07$ ), and genotype-mutagen interaction was not significant in general ( $F = 1.27$ ;  $F_{0.05} = 3.20$ ;  $P = 0.08$ ). In pairwise comparison, we found no significant difference between water and DAB 0.1% for varieties Balaton ( $F = 4.12$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ) and Nyva Odeska ( $F = 4.17$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ), for the concentrations of DAB 0.1% and 0.2% for varieties Nyva Odeska ( $F = 3.09$ ;  $F_{0.05} = 4.99$ ;  $P = 0.07$ ) and Pochaina ( $F = 3.16$ ;  $F_{0.05} = 4.99$ ;  $P = 0.07$ ), between DAB 0.2% and 0.3% for varieties Zelenyi Hai ( $F = 3.22$ ;  $F_{0.05} = 4.99$ ;  $P = 0.07$ ), Nyva Odeska ( $F = 3.78$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ), Borovytsia ( $F = 3.99$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ), Kalancha ( $F = 4.19$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ), Polianka ( $F = 4.11$ ;  $F_{0.05} = 4.99$ ;  $P = 0.06$ ), Pochaina ( $F = 2.77$ ;  $F_{0.05} = 4.99$ ;  $P = 0.08$ ). Genotype-mutagen interaction was significant in negative sense for varieties Nyva Odeska ( $F = 9.14$ ;  $F_{0.05} = 3.55$ ;  $P = 0.03$ ) and Pochaina ( $F = 6.18$ ;  $F_{0.05} = 3.55$ ;  $P = 0.04$ ), having no positive effect. Therefore, in general, DAB as mutagen in combination with these varieties cannot be used for high mutation-inducing complex in any concentration. For the most genotypes, the critical value of the mutagen in terms of induced biodiversity lines was DAB 0.1–0.2%.

As for NEU action, for water and families (lines), after the mutagen action, 12,000 families in the second-third generations were studied and 452 mutant lines were selected (Table 2). As for positive changes resulting from NEU action, the same types at the same stages of the experiment were assessed. Number of valuable forms was 276, and 104 lines with positive changes without any negative additional traits were finally selected for next stages of the breeding process. The number of families was 500 for all the variants. In general, NEU was interesting as a mutagen for breeding practice regarding number of mutations by such parameters as short stem ( $F = 3.49$ ;  $F_{0.05} = 2.55$ ;  $P = 0.04$ ), disease tolerance ( $F = 4.71$ ;  $F_{0.05} = 2.55$ ;  $P = 0.03$ ), productivity ( $F = 2.66$ ;  $F_{0.05} = 2.55$ ;  $P = 0.05$ ), higher protein content ( $F = 4.54$ ;  $F_{0.05} = 2.55$ ;  $P = 0.03$ ), positive changes in protein components ( $F = 7.17$ ;  $F_{0.05} = 2.55$ ;  $P = 0.01$ ), and positive changes in microelement content ( $F = 6.99$ ;  $F_{0.05} = 2.55$ ;  $P = 0.01$ ). As we can see, NEU was more effective for biochemical changes.

**Table 2**  
Rate of positive changes forth – fifth generations, NEU ( $x \pm SD$ ,  $n = 500$ )

Variant	Rate of positive changes, %	Part from general rate, %	Number of lines, pcs	Number of perspective lines	Rate of perspective lines, %
Balaton	0.20 ± 0.03 <sup>a</sup>	0.50	1	0	0.00 ± 0.00 <sup>a</sup>
Balaton, NEU 0.010%	3.20 ± 0.25 <sup>b</sup>	0.70	14	5	1.00 ± 0.17 <sup>b</sup>
Balaton, NEU 0.025%	4.00 ± 0.31 <sup>c</sup>	0.57	17	6	1.20 ± 0.22 <sup>b</sup>
Zoloto Ukrainy	0.60 ± 0.10 <sup>a</sup>	0.75	3	1	0.20 ± 0.09 <sup>a</sup>
Zoloto Ukrainy, NEU 0.010%	3.20 ± 0.25 <sup>b</sup>	0.80	15	7	1.40 ± 0.14 <sup>b</sup>
Zoloto Ukrainy, NEU 0.025%	4.20 ± 0.38 <sup>c</sup>	0.66	19	8	1.60 ± 0.19 <sup>b</sup>
Zelenyi Hai	0.40 ± 0.09 <sup>a</sup>	0.67	2	0	0.00 ± 0.00 <sup>a</sup>
Zelenyi Hai, NEU 0.010%	2.80 ± 0.25 <sup>b</sup>	0.58	13	7	1.40 ± 0.22 <sup>b</sup>
Zelenyi Hai, NEU 0.025%	3.40 ± 0.29 <sup>b</sup>	0.47	15	7	1.40 ± 0.21 <sup>b</sup>
Nyva Odeska	0.60 ± 0.11 <sup>a</sup>	0.50	3	0	0.00 ± 0.00 <sup>a</sup>
Nyva Odeska, NEU 0.010%	2.40 ± 0.24 <sup>b</sup>	0.63	11	5	1.00 ± 0.19 <sup>b</sup>
Nyva Odeska, NEU 0.025%	4.40 ± 0.39 <sup>c</sup>	0.65	19	9	1.80 ± 0.25 <sup>c</sup>
Borovytsia	0.40 ± 0.11 <sup>a</sup>	0.40	1	0	0.00 ± 0.00 <sup>a</sup>
Borovytsia, NEU 0.010%	3.20 ± 0.34 <sup>b</sup>	0.73	14	3	0.60 ± 0.17 <sup>b</sup>
Borovytsia, NEU 0.025%	4.20 ± 0.38 <sup>c</sup>	0.68	18	5	1.00 ± 0.21 <sup>b</sup>
Kalancha	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Kalancha, NEU 0.010%	3.80 ± 0.38 <sup>b</sup>	0.86	17	4	0.80 ± 0.18 <sup>b</sup>
Kalancha, NEU 0.025%	4.40 ± 0.43 <sup>b</sup>	0.59	19	4	0.80 ± 0.19 <sup>b</sup>
Polianka	0.20 ± 0.08 <sup>a</sup>	0.50	1	0	0.00 ± 0.00 <sup>a</sup>
Polianka, NEU 0.010%	3.80 ± 0.33 <sup>b</sup>	1.12	17	7	1.40 ± 0.27 <sup>b</sup>
Polianka, NEU 0.025%	5.20 ± 0.45 <sup>c</sup>	0.96	22	11	2.20 ± 0.32 <sup>b</sup>
Pochaina	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Pochaina, NEU 0.010%	4.00 ± 0.36 <sup>b</sup>	1.11	18	7	1.40 ± 0.26 <sup>b</sup>
Pochaina, NEU 0.025%	4.00 ± 0.35 <sup>b</sup>	0.71	17	8	1.60 ± 0.29 <sup>b</sup>

Note: see Table 1.

The rate of positive changes induced by NEU action was higher after using higher concentrations of the mutagen in varieties Balaton, Zoloto Ukrainy, Nyva Odeska, Borovytsia, Polianka, in all the variants. For all the varieties, difference between variant without mutagen action and first concentration was significant according to ANOVA. For

varieties Zelenyi Hai ( $F = 3.22$ ;  $F_{0.05} = 5.17$ ;  $P = 0.08$ ), Kalancha ( $F = 3.45$ ;  $F_{0.05} = 5.17$ ;  $P = 0.08$ ), Pochaina ( $F = 3.98$ ;  $F_{0.05} = 5.17$ ;  $P = 0.07$ ), there were no differences between the first and second concentrations. Rate of positive changes in all the varieties (up to 4.0–4.4%, without significant difference) was on the same level, except Zelenyi Hai (up to

3.4%) ( $F = 6.33$ ;  $F_{0.05} = 5.17$ ;  $P = 0.04$ ). Therefore, by this parameter, the variety Zelenyi Hai exerted a lower level of variability from NEU in obtaining of valuable forms. By the factor analysis, induction of the overall rate of positive changes was affected by increase in the concentrations of NEU ( $F = 11.01$ ;  $F_{0.05} = 2.49$ ;  $P = 0.002$ ), but not for differences in the initial variety ( $F = 2.55$ ;  $F_{0.05} = 4.11$ ;  $P = 0.08$ ). Genotype-mutagen interaction was significant only for variety Zelenyi Hai ( $F = 5.53$ ;  $F_{0.05} = 3.80$ ;  $P = 0.04$ ).

The share from general rate was not higher than for DAB, about 0.5–0.8% for the most variants, except Polianka and Pochaina (up to 1.1%). There were no other statistically significant correlations with changes in concentrations or initial forms according to this indicator. Number of promising lines shows suitability of all concentrations of NEU for induction of new valuable lines, especially for variety Polyanka. A very valuable indicator for the breeding process was for rate of promising lines (from general number of families, 500 in all cases), without statistically significant difference between the first and second concentrations, except Nyva Odeska ( $F = 6.05$ ;  $F_{0.05} = 5.17$ ;  $P = 0.04$ ), but the rate of promising lines was influenced by increase in the concentrations of NEU ( $F = 4.12$ ;  $F_{0.05} = 2.49$ ;  $P = 0.003$ ) and no differences by the genotypes ( $F = 3.11$ ;  $F_{0.05} = 4.11$ ;  $P = 0.06$ ), genotype-mutagen interaction was not significant in general as well ( $F = 1.99$ ;  $F_{0.05} = 3.55$ ;  $P = 0.08$ ), except Polianka ( $F = 6.25$ ;  $F_{0.05} = 3.55$ ;  $P = 0.03$ ). Pairwise comparison revealed significant difference between water and NEU 0.010% for all varieties. For the concentrations of NEU 0.010% and NEU 0.025% for all varieties, no significant

difference was found, except for Nyva Odeska ( $F = 6.76$ ;  $F_{0.05} = 5.17$ ;  $P = 0.04$ ). For each variety, the genotype-mutagen interaction was significantly positive Polianka ( $F = 6.90$ ;  $F_{0.05} = 3.55$ ;  $P = 0.03$ ) and negatively for Kalancha ( $F = 7.17$ ;  $F_{0.05} = 3.55$ ;  $P = 0.03$ ). Therefore, in general, NEU as mutagen in combination with Polyanka can be used for high mutation induction in all concentrations. For the most genotypes the critical value of the mutagen in terms of induced biodiversity lines in the range of NEU 0.010–0.025%.

Action of NMU was more similar to NEU, but not for DAB, with more variability according to the same traits. For water and families (lines) after mutagen action, 11,950 families in the second-third generations were examined and 573 mutant lines were selected (Table 3). As for positive changes from NMU action, the same types at the same stages of the study as for previous two mutagens were estimated. Number of valuable forms was 247 and 63 lines with positive changes without any negative additional traits were finally selected for next stages of the breeding process. The number of families was 500 for all variants, except Zelenyi Hai, NMU 0.025%. In general, NMU was interesting as mutagen for the breeding practice regarding number of mutations by parameters such as short-stem ( $F = 4.67$ ;  $F_{0.05} = 2.55$ ;  $P = 0.03$ ), tolerance to diseases ( $F = 7.89$ ;  $F_{0.05} = 2.55$ ;  $P = 0.002$ ), positive changes in protein components ( $F = 5.11$ ;  $F_{0.05} = 2.55$ ;  $P = 0.03$ ), positive changes in microelement content ( $F = 4.14$ ;  $F_{0.05} = 2.55$ ;  $P = 0.04$ ). As we can see, NMU was not so effective as NEU or DAB with one positive moment for induction of forms with tolerance to diseases.

**Table 3**

Rate of positive changes forth – fifth generations, NMU ( $x \pm SD$ ,  $n = 500$ , for Zeleny Gai, NMU 0.025%  $n = 450$ )

Variant	Rate of positive changes, %	Part from general rate, %	Number of lines	Number of promising lines	Rate of promising lines, %
Balaton	0.20 ± 0.05 <sup>a</sup>	0.00	1	0	0.00 ± 0.00 <sup>a</sup>
Balaton, NMU 0.0125%	3.60 ± 0.38 <sup>b</sup>	0.60	15	3	0.60 ± 0.15 <sup>b</sup>
Balaton, NMU 0.025%	4.20 ± 0.43 <sup>b</sup>	0.45	16	4	0.80 ± 0.19 <sup>b</sup>
Zoloto Ukrainy	0.60 ± 0.10 <sup>a</sup>	0.75	2	0	0.00 ± 0.00 <sup>a</sup>
Zoloto Ukrainy, NMU 0.0125%	4.00 ± 0.37 <sup>b</sup>	0.77	17	3	0.60 ± 0.17 <sup>b</sup>
Zoloto Ukrainy, NMU 0.025%	3.80 ± 0.31 <sup>b</sup>	0.46	15	5	1.00 ± 0.24 <sup>b</sup>
Zelenyi Hai	0.40 ± 0.10 <sup>a</sup>	0.67	2	0	0.00 ± 0.00 <sup>a</sup>
Zelenyi Hai, NMU 0.0125%	3.00 ± 0.35 <sup>b</sup>	0.50	13	4	0.80 ± 0.14 <sup>b</sup>
Zelenyi Hai, NMU 0.025%	4.20 ± 0.38 <sup>c</sup>	0.43	14	4	0.89 ± 0.18 <sup>b</sup>
Nyva Odeska	0.60 ± 0.16 <sup>a</sup>	0.50	2	0	0.00 ± 0.00 <sup>a</sup>
Nyva Odeska, NMU 0.0125%	2.80 ± 0.28 <sup>b</sup>	0.64	11	3	0.60 ± 0.19 <sup>b</sup>
Nyva Odeska, NMU 0.025%	4.00 ± 0.39 <sup>c</sup>	0.47	15	5	1.00 ± 0.23 <sup>b</sup>
Borovytsia	0.40 ± 0.08 <sup>a</sup>	0.40	2	0	0.00 ± 0.00 <sup>a</sup>
Borovytsia, NMU 0.0125%	3.00 ± 0.34 <sup>b</sup>	0.56	13	4	0.80 ± 0.17 <sup>b</sup>
Borovytsia, NMU 0.025%	3.80 ± 0.38 <sup>b</sup>	0.48	15	6	1.20 ± 0.22 <sup>b</sup>
Kalancha	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Kalancha, NMU 0.0125%	4.60 ± 0.38 <sup>b</sup>	0.72	20	4	0.80 ± 0.17 <sup>b</sup>
Kalancha, NMU 0.025%	5.20 ± 0.43 <sup>b</sup>	0.48	19	5	1.00 ± 0.21 <sup>b</sup>
Polianka	0.20 ± 0.07 <sup>a</sup>	0.50	1	0	0.00 ± 0.00 <sup>a</sup>
Polianka, NMU 0.0125%	3.00 ± 0.23 <sup>b</sup>	0.71	12	3	0.60 ± 0.15 <sup>b</sup>
Polianka, NMU 0.025%	3.60 ± 0.27 <sup>b</sup>	0.56	15	4	0.80 ± 0.19 <sup>b</sup>
Pochaina	0.00 ± 0.00 <sup>a</sup>	0.00	0	0	0.00 ± 0.00 <sup>a</sup>
Pochaina, NMU 0.0125%	3.20 ± 0.36 <sup>b</sup>	0.73	14	3	0.60 ± 0.13 <sup>b</sup>
Pochaina, NMU 0.025%	3.00 ± 0.30 <sup>b</sup>	0.43	13	3	0.60 ± 0.14 <sup>b</sup>

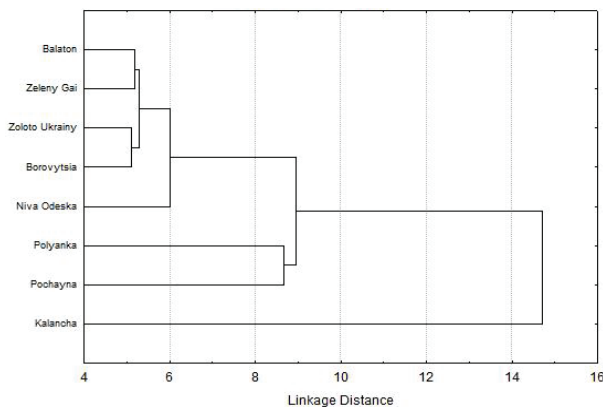
Note: see Table 1.

The rate of positive changes from NMU action was higher with growing concentrations for varieties Zelenyi Hai and Nyva Odeska, in all variants. For all the varieties, difference between variant without mutagen action and first concentration was significant according to ANOVA. For varieties Balaton ( $F = 5.08$ ;  $F_{0.05} = 5.17$ ;  $P = 0.06$ ), Zoloto Ukrainy ( $F = 4.98$ ;  $F_{0.05} = 5.17$ ;  $P = 0.06$ ), Nyva Odeska ( $F = 3.77$ ;  $F_{0.05} = 5.17$ ;  $P = 0.07$ ), Borovytsia ( $F = 4.55$ ;  $F_{0.05} = 5.17$ ;  $P = 0.06$ ), Kalancha ( $F = 3.99$ ;  $F_{0.05} = 5.17$ ;  $P = 0.07$ ), Polianka ( $F = 3.88$ ;  $F_{0.05} = 5.17$ ;  $P = 0.07$ ), and Pochaina ( $F = 3.14$ ;  $F_{0.05} = 5.17$ ;  $P = 0.08$ ), there were no differences between the first and second concentrations. Rate of positive mutation changes can be divided into several groups. The first group included Balaton, Zoloto Ukrainy, Zelenyi Hai and Nyva Odeska, Borovytsia (up to 3.8–4.2%), second Polianka and Pochaina (up to 3.2–3.6%), and third Kalancha (up to 5.2%). Thus, by this parameter, Kalancha showed more variability for NMU in obtaining valuable forms. According to the factor analysis, induction of the overall rate of positive changes was affected by increase in the concentrations of NMU ( $F = 4.51$ ;  $F_{0.05} = 2.49$ ;  $P = 0.04$ ), but not for

differences in the initial variety ( $F = 3.95$ ;  $F_{0.05} = 4.11$ ;  $P = 0.06$ ). Genotype-mutagen interaction was significant for varieties Zelenyi Hai ( $F = 7.13$ ;  $F_{0.05} = 3.80$ ;  $P = 0.02$ ), Polianka ( $F = 5.00$ ;  $F_{0.05} = 3.80$ ;  $P = 0.04$ ), and Pochaina ( $F = 4.93$ ;  $F_{0.05} = 3.80$ ;  $P = 0.04$ ).

The share in general rate was not higher than for DAB, about 0.5–0.7% for the most variants. Rate of promising lines (from general number of families, 500 in total, only one variant Zelenyi Hai, NMU 0.025% 450), without statistically significant difference between the first and second concentrations, but the rate of promising lines was influenced by increase in the concentrations of NMU ( $F = 3.90$ ;  $F_{0.05} = 2.49$ ;  $P = 0.003$ ). There were no differences by genotypes ( $F = 3.29$ ;  $F_{0.05} = 4.11$ ;  $P = 0.06$ ), and genotype-mutagen interaction was not significant in general as well. For each variety, the genotype-mutagen interaction was significant in positive sense for varieties Polianka ( $F = 5.00$ ;  $F_{0.05} = 3.55$ ;  $P = 0.04$ ), Kalancha ( $F = 4.17$ ;  $F_{0.05} = 3.55$ ;  $P = 0.04$ ), and Pochayna ( $F = 3.87$ ;  $F_{0.05} = 3.55$ ;  $P = 0.05$ ). Thus, in general, NMU as mutagen in combination with Polyanka, Kalancha, and Pochayna can be used for mutation induction in all concentrations.

In accordance with the action of mutagens of different nature, a cluster analysis was conducted to take into account the complex response to the site-specific action of all the genotypes (Fig. 1).



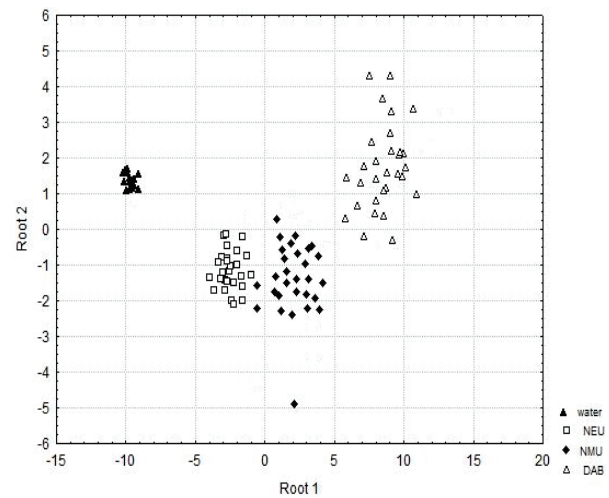
**Fig. 1.** Results of cluster analysis by rate of positive changes: single linkage, Euclidean distance

Three groups were identified in total. The first ones included Balaton, Zoloto Ukrainy, Nyva Odeska, and Borovytsia, which generally did not react differently to any of the applied mutagens. We conclude that the use of these genotypes to create a high-performance mutagen-initial form system is impossible for substances with low damaging potential. This does not mean that the application of this group will not lead to agriculturally valuable lines, but the effectiveness of this process will have certain average values.

In the second group, there is one variety Kalancha, which exhibited a specific reaction to all three mutagens, and obviously, according to useful features of the genotype structure, the use of these factors will lead to the strengthening of the mutation process. The third group consists of two varieties Polianka and Pochayna. Considering the peculiarities of the action of these substances, a relatively large number of forms with biochemical and physiological micro-changes should be expected.

The results of the discriminant analysis conducted for the mutagens (Fig. 2) revealed that NEU and NMU actually showed the same effectiveness in terms of effect on the entire group of genotypes. The effect of

DAB was the most variable and, apparently, DAB as a mutagen had a more continuous nature of action than the other factors and depended slightly on the specific subject of action in the range of the applied material.



**Fig. 2.** Results of discriminant analysis for mutagens: single linkage, Euclidean distance

In the spectrum of changes obtained, the discriminant analysis divided the traits were divided as more or less changeable in accordance with nature of mutagen. A classification analysis was also carried out both for individual characteristics of the mutation process (Tables 1–3) and for genotypes (varieties) (Table 4). For DAB mutations of such traits as short stem, long spike, early ripeness, and tillering capacity were significant. As mutagen, this DAB was less effective for this initial material. For NEU, more typical changes were short stem, early ripeness, productivity, tillering capacity, higher protein content, positive changes in protein components, and positive changes in microelement content. For NMU, those changes were short stem, early ripeness, tolerance to diseases, productivity, tillering capacity, higher protein content, positive changes in protein components, and positive changes in microelement content.

**Table 4**  
Positive changed traits. Results of discriminant analyzes

Trait	Wilks Lambda $\lambda$	Mutagens at model	F <sub>0.05</sub>	P
Thick stem	0.331	no	1.91	0.092
Short-stem	0.084	DAB, NEU, NMU	17.34	0.001
Semi-dwarf	0.221	no	3.98	0.084
Dwarf	0.260	no	3.11	0.085
Intense epicuticular wax accumulation	0.230	no	3.72	0.097
Large-size grain	0.223	no	3.88	0.084
Long spike	0.114	DAB	7.14	0.003
Large-size spike	0.296	no	2.34	0.095
Early ripeness	0.123	DAB, NEU, NMU	11.04	0.001
Tolerance for diseases	0.144	NMU	7.67	0.002
Productive	0.159	NEU	4.57	0.054
Tillering capacity	0.144	DAB, NEU, NMU	4.90	0.042
Higher protein content	0.130	NEU	5.01	0.044
Positive changes at protein components	0.154	NEU, NMU	4.95	0.043
Positive changes at microelement content	0.143	NEU, NMU	7.68	0.002

Note: DAB – 1,4-bisdiazoacetylbutane, NEU – nitrosomethylurea, NMU – nitrosomethylurea.

As can see, NEU and NMU were more similar in these actions. At the same time, DAB was effective only for induction long spike mutation forms and NEU and NMU were effective for short stem, early ripeness, tolerance to diseases, tillering capacity, positive changes at protein components, positive changes at microelement content. Substance NMU was more effective for inducing mutations with high disease tolerance, NEU for mutants with high protein content and productive lines. No mutagen effectively induced such traits as thick stem semi-dwarf, dwarf, intense epicuticular-wax accumulation, large-size grain forms. As conclusion, action of NEU and NMU in complex was the most suitable variant for the breeding process. Among the more productive lines, we found all varieties except Nyva Odeska as initial

material. Three varieties Balaton, Zoloto Ukrainy, Zelenyi Hai were represented by forms resulted from the action of NEU alone. Four other varieties were represented mainly by two or three lines under the action of NEU and NMU. In only one case, DAB was effective as a factor inducing productive lines and only for the Kalancha variety. All selected lines ultimately exceeded the standard based on the results of three years of testing, but were not equal to each other in this excess.

Therefore, in 2021, the lines were divided into three groups based on exceeding the standard – lines 213, 278, 311, 415 made up the group of the most productive lines, forms 401 and 442, 467 attained the intermediate position, lines 17, 29, 224, 345, 389 exceeded the standard in

terms of yield, and they were significantly inferior to the first group. In 2022, differentiation was significantly higher. Lines 17, 278, 311, 415, 442 stood out in a similar first group; the remaining three groups had intermediate values. In the third year of testing, the separation equaled approximately the same number of clusters as in the first,

although the year was more favorable for semi-intensive varieties than for high-intensive genotypes. Lines 17, 29, 213, 278, 311, 415, 442 formed a similar group to the first group; forms 224, 401, 467 were on the intermediate position between the two groups of lines, having significant differences in yield.

**Table 5**

Yielding capacity of agrocenosis of mutant lines of common winter wheat during the experiment (DSAEU, 2021–2023, t/ha)

Variant	Origin	2021	2022	2023	Average
1	Podolianka, standard	5.28 ± 0.08 <sup>a</sup>	5.46 ± 0.09 <sup>a</sup>	6.12 ± 0.11 <sup>a</sup>	5.62 ± 0.09 <sup>a</sup>
17	Balaton, NEU 0.010%	6.29 ± 0.14 <sup>b</sup>	7.01 ± 0.16 <sup>c</sup>	6.81 ± 0.17 <sup>b</sup>	6.70 ± 0.15 <sup>b</sup>
29	Zoloto Ukrainy, NEU, 0.0125%	6.31 ± 0.15 <sup>b</sup>	6.59 ± 0.14 <sup>b</sup>	6.89 ± 0.16 <sup>b</sup>	6.60 ± 0.15 <sup>b</sup>
213	Zelenyi Hai, NEU 0.010%	6.91 ± 0.17 <sup>c</sup>	6.38 ± 0.11 <sup>b</sup>	6.23 ± 0.15 <sup>a</sup>	6.51 ± 0.15 <sup>b</sup>
224	Borovytsia, NEU 0.010%	6.51 ± 0.14 <sup>b</sup>	6.29 ± 0.08 <sup>b</sup>	6.15 ± 0.14 <sup>a</sup>	6.32 ± 0.12 <sup>bc</sup>
278	Borovytsia, NMU 0.0125%	6.73 ± 0.14 <sup>c</sup>	7.16 ± 0.13 <sup>c</sup>	6.69 ± 0.17 <sup>b</sup>	6.86 ± 0.16 <sup>b</sup>
311	Kalanča, DAB 0.200%	6.69 ± 0.15 <sup>c</sup>	7.01 ± 0.12 <sup>c</sup>	6.79 ± 0.16 <sup>b</sup>	6.83 ± 0.15 <sup>b</sup>
345	Kalanča, NEU, 0.025%	6.30 ± 0.10 <sup>b</sup>	6.22 ± 0.09 <sup>bd</sup>	6.22 ± 0.18 <sup>a</sup>	6.25 ± 0.14 <sup>c</sup>
389	Polianka, NEU 0.010%	6.29 ± 0.09 <sup>b</sup>	5.92 ± 0.08 <sup>c</sup>	6.17 ± 0.14 <sup>a</sup>	6.13 ± 0.12 <sup>c</sup>
401	Polianka, NEU 0.025%	6.54 ± 0.09 <sup>bc</sup>	6.12 ± 0.09 <sup>de</sup>	6.43 ± 0.15 <sup>c</sup>	6.36 ± 0.11 <sup>bc</sup>
415	Polianka, NMU 0.0125%	6.70 ± 0.15 <sup>c</sup>	7.14 ± 0.09 <sup>c</sup>	6.72 ± 0.14 <sup>bc</sup>	6.85 ± 0.11 <sup>b</sup>
442	Pochaina, NEU 0.010%	6.55 ± 0.11 <sup>bc</sup>	7.15 ± 0.11 <sup>c</sup>	6.89 ± 0.13 <sup>b</sup>	6.86 ± 0.12 <sup>b</sup>
467	Pochaina, NMU 0.0125%	6.53 ± 0.11 <sup>bc</sup>	6.26 ± 0.10 <sup>bd</sup>	6.29 ± 0.16 <sup>ac</sup>	6.36 ± 0.13 <sup>bc</sup>

Note: see Table 1.

Based on the results of three years of testing, three groups of lines were again identified: 17, 29, 213, 278, 311, 415, 442 made up the group of the most productive lines ( $F = 8.92$ ;  $F_{0.05} = 4.11$ ;  $P = 0.02$ ), forms 224, 401, 467 took t intermediate position, lines 345, 389, which exceeded the standard in terms of yield ( $F = 12.14$ ;  $F_{0.05} = 3.55$ ;  $P = 0.005$ ), were significantly inferior to the first group ( $F = 9.32$ ;  $F_{0.05} = 3.90$ ;  $P = 0.01$ ). At the same time, lines 278, 311, 415 were stable regarding the trait, whereas the rest varied quite significantly from year to year. In terms of technological qualities, such lines as 345, 401, and 415 stood out positively in terms of protein content. Lines 29, 345, 401, 415, 442, and 467 were notable in terms of gluten content, which in general shows that the characteristics are quite closely related. In general, all the obtained forms, except for 311, can be classified as those with good grain quality, and therefore the only productive line produced using DAB cannot be considered promising. In general, the first and second signs had rather low variability.

**Table 6**

Technological properties of wheat grain (DSAEU, 2022)

Variety/ line	Protein, %	Gluten, %	Glutenins		Gliadins
			HMW	LMW	
Podolianka	13.97 <sup>a</sup>	25.14 <sup>a</sup>	0.15993 <sup>a</sup>	0.46342 <sup>a</sup>	0.4572 <sup>a</sup>
17	13.97 <sup>a</sup>	24.99 <sup>a</sup>	0.16443 <sup>a</sup>	0.49435 <sup>b</sup>	0.4565 <sup>a</sup>
29	14.12 <sup>a</sup>	26.23 <sup>b</sup>	0.16245 <sup>a</sup>	0.48453 <sup>b</sup>	0.4453 <sup>a</sup>
213	14.01 <sup>a</sup>	25.15 <sup>a</sup>	0.16353 <sup>a</sup>	0.46467 <sup>a</sup>	0.4425 <sup>ab</sup>
224	13.89 <sup>a</sup>	25.16 <sup>a</sup>	0.17356 <sup>b</sup>	0.46465 <sup>a</sup>	0.4131 <sup>c</sup>
278	13.97 <sup>a</sup>	25.54 <sup>a</sup>	0.16435 <sup>a</sup>	0.46100 <sup>a</sup>	0.4424 <sup>ab</sup>
311	13.17 <sup>b</sup>	22.17 <sup>c</sup>	0.15231 <sup>a</sup>	0.45454 <sup>a</sup>	0.4657 <sup>a</sup>
345	14.67 <sup>c</sup>	27.78 <sup>d</sup>	0.19523 <sup>c</sup>	0.42313 <sup>c</sup>	0.4856 <sup>b</sup>
389	13.03 <sup>b</sup>	23.23 <sup>c</sup>	0.15022 <sup>a</sup>	0.50760 <sup>b</sup>	0.4500 <sup>a</sup>
401	14.52 <sup>c</sup>	27.19 <sup>d</sup>	0.20453 <sup>b</sup>	0.47300 <sup>ad</sup>	0.4948 <sup>b</sup>
415	14.45 <sup>c</sup>	27.78 <sup>d</sup>	0.21453 <sup>bd</sup>	0.46999 <sup>a</sup>	0.4048 <sup>c</sup>
442	14.22 <sup>bc</sup>	27.12 <sup>d</sup>	0.16444 <sup>a</sup>	0.41545 <sup>c</sup>	0.4148 <sup>c</sup>
467	14.01 <sup>a</sup>	26.34 <sup>b</sup>	0.15789 <sup>a</sup>	0.45934 <sup>a</sup>	0.4848 <sup>b</sup>

Note: see Table 1.

From the point of view of grain quality, positive traits are high presence of high-molecular-weight glutenins (the trait is highly variable), small presence of low-molecular-weight glutenins, and high content of gliadin (moderately variable) in the composition of wheat proteins. The first group included lines 224, 345, 401, 415, the second 345, 442, the third 345, 401, 467. Therefore, we can conclude that line 345 had a complete combination of positive qualities. From group of consistently high-yielding genotypes, line 415 showed the best combination with high grain quality (high protein, gluten, high-molecular-weight glutenins), and 311 drops out due to its low grain quality.

## Discussion

The regularity of the beneficial mutational process has been described (Lal et al., 2020; Nazarenko et al., 2021), which makes using this

type of variability more manageable, reliable and predictive in order to produce new material with required potential (Nazarenko et al., 2022).

Mutations are a reliable and permanent source of local genetic resources (Hongjie et al., 2019), and therefore producing high-intensity lines with a long grained spike (Hongjie et al., 2019) of early ripeness (Ergün et al., 2023; Kartseva et al., 2023) based on local material is quite possible and regular (Cann et al., 2022).

Based on the obtained material, it can be concluded that it is advisable to use mainly mutagens of NEU and NMU for the local material (Chakraborty et al., 2023). The results demonstrate that the use of these agents as a mutagenic factor should be used primarily to obtain forms as components for the subsequent improvement of existing varieties through recombinant breeding (Ahumada-Flores et al., 2021; le Roux et al., 2021). It is much less likely to produce lines that can be used directly as commercial varieties (Shimelis et al., 2019).

As a mutagenic factor, NEU and NMU exerted more significant dependence on the peculiarities of the variety-mutagenic interaction, i.e., depends on DNA structure of the initial variety with a clear subdivision according to genetically determined reactivity to the action of this mutagenic factor. At the same time, DAB was not that site-specific. The yield lines obtained under the action of NEU and NMU in some cases had positive changes in grain-quality traits (Nazarenko et al., 2019), which is very suitable for the country's agriculture and leads to increase in grain quality (Beiko & Nazarenko, 2022).

The patterns observed in the study also make it possible to show that a fairly significant number of traits that are undoubtedly economically valuable changed insignificantly under the influence of the studied mutagenic factors. This once again indicates that in the case of chemical supermutagens, a lot depends on the well-chosen starting material depending on specific mutagen, since these characteristics generally showed quite high variability in a number of previous studies, especially when using more active chemical mutagens (le Roux et al., 2021; OlaOlorun et al., 2021).

We note that again, with increase in the concentration of mutagenic substances, the risk of combination of negative and positive changes increases significantly. The number of such cases is quite high even for moderately active substances, which is why up to half of all obtained promising mutant forms are ignored (Mangi et al., 2021; Hassine et al., 2023). To some extent, this, of course, also depends on genotype of the source material, and varieties that showed high variability in terms of the effects of mutagenic depression in the first generation and in terms of the frequency and spectrum of hereditary changes in the second or third generations, are not always the most suitable for use (Abaza et al., 2020). At the same time, in these experiments the advantage was the production of economically valuable forms, the most promising as future varieties, specifically for genotypes that are more resistant to negative impacts (Ram et al., 2019; Ahumada-Flores et al., 2020).

It should also be noted that there was a stronger relationship between the agriculturally valuable traits and the rate of valuable mutations obtained for these genotypes and the general rate and spectra



of visually identified mutations. This pattern has been developed in terms of the past experiments, but not for chemical mutagenesis (Yali & Mitiku, 2022).

We note that despite the low probability of obtaining a mutant line with high properties, which can be directly used as a commercial variety in the future, the general possibilities of mutagenesis and the use of these substances are quite high in inducing improvements in certain key economically valuable traits (Ariraman et al., 2018; OlaOlorun et al., 2021). In general, the induction of changes in biochemical parameters turns out to be quite low, especially in terms of reducing the content of low-molecular glutenins, which are unfavorable for the bread-making industry, and increasing the content of valuable microelements, which are clearly deficient in wheat grain, which significantly affects its nutritional value (Spencer-Lopes et al., 2018).

## Conclusions

Local genetic materials (three varieties) were quite favorable initial forms for the creation of high-yielding and high-quality lines with positive changes in plant structure, time of critical plant-development phases, content and structure of protein and microelement complex. While combined with the proper mutagens (first of all NEU and NMU in optimal concentrations) that product high-value breeding variabilities with a sufficiently higher rate, genetic (as future parent components) and breeding (as future commercial varieties) valuable forms can be induced with more satisfactory results. It should be taken into account that the key valuable morphometrics traits (like plant and spike structure) and characteristics of development are easier to change than the technological properties of grain (such as protein structure and content in value microelements). Therefore, it is still advisable to pay more attention to winter-wheat genetic improvement when assessing the initial forms during the first stages of selection (second-third generations). Classical crossbreeding methods for genetic improvement with the mutational process as a source for new valuable parent form remain effective for future creation of high-yield and high grain-quality forms. Our further researches will focus on valuable traits such as drought tolerance and winter hardiness (which is assessed by changes in the photosynthetic activity in critical phases and parameters of plant survival in winter period), peculiarities of the use and accumulation of mineral substances for the studied lines to confirm the parameters that provide the identified advantage in terms of grain-yielding capacity and quality traits and its relations with growth conditions. It is also planned to study high-active mutagens inducing new forms based on the same local and international varieties and for the same traits.

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