



Original researches

Received: 09.01.2023
Revised: 12.02.2023
Accepted: 14.02.2023

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Cite this article: Horshchar, V., &
Nazarenko, M. (2023). Features of
the action of a highly active chemical
agent for new winter wheat
genotypes. *Agrology*, 6(1), 15–20.
doi: 10.32819/021203

Features of the action of a highly active chemical agent for new winter wheat genotypes

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Abstract. The use of strong ecogenetic factors leads to incompletely predictable complex changes, requiring a detailed consideration of the mechanisms of their interaction with the subjects (genotypes) of the mutagenic impact, the relationship between individual indicators of variability, the nature of the chemical agent, its concentration, and genotype of the source material. Winter wheat seeds of eight varieties (Balaton, Borovytsia, Zelenyi Hai, Zoloto Ukrainy, Kalancha, Nyva Odeska, Polianka, Pochaina) were subjected by DMS (dimethylmethansulfate) in 0.0125%, 0.0250%, and 0.0500% concentrations in water solution for 24 hours. The overall frequency and range of altered forms were analyzed, and the inheritance of the identified changes was verified in the subsequent generations. The studies have demonstrated that two varieties Zelenyi Hai and Kalancha are promising for further research in terms of the variability characteristics to optimize the yield of mutant forms. Depending on the indicators of variability, the response of other varieties in general demonstrated a plateau or a decrease in the variability with increasing concentration in the range of 0.025–0.050%, or the variability decreased significantly. In general, as an indicator of induced diversity, the level of variability, which reflects not only quantitative but also qualitative characteristics of the mutation process, is more effective. In most cases, the use of DMS within 0.025% is optimal. The mutagen causes a large number of useful changes such as short stature, productivity, early ripeness, which are model for the mutation process. However, they are often complex, occurring with negative mutations. In addition, undesirable is the high number of tall-stalked and sterile forms, which limits the practical application of this agent. It is planned to analyze hidden (micromutational) biochemical and physiological changes in the obtained lines, primarily in terms of resistance to abiotic factors, greater nutritional usefulness of the obtained forms in terms of the content of valuable microelements and protein components, clarifying the complexity of the observed changes and linking them with those recorded earlier.

Keywords: winter wheat; mutation rate; dimethylmethansulfate; level of changeability; mutations; ecogenetics.

Introduction

Ecological and genetic variability is the basis for building stably functioning, highly productive reproducible agrocenoses of grain crops. At the same time, as an ecological component, we have a change in the adaptability of the obtained forms and communities, the features of the manifestation of new signs and properties in specific ecological environments, the ecological-geographical method of selecting source material to produce new forms (Beiko & Nazarenko, 2022). The genetic component is responsible for the level of variability, or rather the range of possible changes in both individual genes and their associations, features of implementation of new properties (Gorbatova et al., 2020), pattern of complex interaction between the changed and the wild component of the original genotype (Von Well et al., 2022).

The instability of modern wheat varieties is due to the superselectivity of their germ plasm, which often also determines the low adaptability of the material used (Ahumada-Flores et al., 2021). At the same time, many local forms are of a semi-intensive nature and initially are ecotypically designed for rather high adaptability, especially to local conditions (Dyulgerova & Dyulgerov, 2020), but their use in combination with high-intensity agricultural technologies is meaningless due to a rather low genetically determined boundary of the implementation of economically valuable traits (Anter, 2021).

With these introductory data, the role of the ecogenetic factor as an agent of variability appears in the possibility of quickly adjusting forms of an intensive ecotype to local conditions and in improving individual traits (while leaving the rest in their original form) of local varieties (Abdelsalam et al., 2019; Chernysky & Gumentyk, 2020). However, while the first case is more focused on the use of low doses and

concentrations for the gradual transformation of the source material, the other is more focused on the intensification of local forms (Bondarenko & Nazarenko, 2020). Of course, two points must be taken into account (Dwinanda et al., 2020). The first one is related to the fact that such changes in general are always complex and it is quite difficult to count on receiving only neutral-positive shifts (Ahumada-Flores et al., 2020). However, this aspect can be rather easily regulated by a large amount of diverse starting material and a larger sample of it (Ergün et al., 2023), which is already a key condition for the successful use of experimental mutagenesis (Chaudhary et al., 2019; Hong et al., 2022).

The second aspect is genetically determined mechanisms of resistance to the effects of individual mutagens and mutational pressure in general (Bezie et al., 2020). The use of chemical supermutagens significantly simplifies overcoming the first barrier (El-Mouhamady et al., 2020; Anter, 2021), whereas the site-specific action leads to big problems for this type of substances due to a more precise and more diverse interaction with the subject's DNA (Nazarenko, 2020).

At the same time, it is possible to find forms, primarily among local genetic diversity, with a higher responsiveness just to the site-specific action of individual chemical agents (Jalal et al., 2021), primarily at moderate concentrations (Abdelsalam et al., 2019). Considering that such forms have already been reliably identified for DMS-related compounds (Bilgin et al., 2022), we should expect the same for DMS itself. Such combinations make it possible to increase the yield of valuable forms up to 180% only due to peculiarities of the genome architecture of the starting material and correctly chosen concentrations. It is extremely important that the selected material also belongs to more intensive varieties, since otherwise changes in complex micromutations may not be substantial enough for the local material to reach indicators of the Western European ecotype (Von Well et al., 2023).

The main purpose of our research was to demonstrate the features of variability and induced biodiversity in the modern winter wheat genotypes exposed to a mutagen with a high damaging ability, the ability of this agent to result in new forms with different groups of traits, the effect of the genotype of the subject of mutagenic exposure, the nature of influence of this factor and its dose on the total frequency and the level of variability, in general and for individual traits and groups of traits. It was also important to identify the threshold concentrations, the possible presence of a plateau in the impact of the mutagen, and the relationship of these indicators with the genotype of the source material.

Materials and methods

The experiment has been conducted under the conditions of the experimental fields of the Research-Education Center of the Dnipro State Agrarian Economic University in 2017–2021.

Winter wheat seeds (1,000 grains for each concentration and water) were exposed to DMS (dimethylmethansulfate) 0.0125%, 0.0250%, 0.0500% (Sigma-Aldrich, Germany). The seeds were exposed for 24 hours, according to the generally recommended method for the protocol of using chemical mutagens. These concentrations are crucial for mutagens (chemical supermutagens) of this group. The control was soaked in water (Spencer-Lopes et al., 2018; Nazarenko, 2020).

The seed samples were sown in 32 variants (in total) (10-row plots for every variant, in water as control, interrow-spacing was 0.15 m, length of a row was 1.5 m) by varieties (ecotypes in brackets FS – forest-steppe, all - for all zones, S – steppe) Balaton (FS), Borovytsia (all), Zelenyi Hai (S), Zoloto Ukrainy (FS), Kalancha (all), Nyva Odeska (all), Polianka (all), Pochaina (all). The genotypes were identified according to the variability for North Steppe subzone (Dnipro region).

The agrotechnology of crop cultivation is crucial for the Steppe zone (semi-arid area).

The sowing material was cultivated in rows with inter and intra-row spacing of 50 and 30 cm, respectively, for first generation of families. The control was sample of non-treated grains of parent varieties and plants were also grown after each ten rows for each variant as comparison with the first-generation plants and next-generation families. Mutant lines and group rows were planted in three repetitions with control rows of parent variety for each twenty-row plot (Mangi et al., 2021).

Mutation families from At M2–M3 generations were selected by visual assessment. The sowing was done manually in the late September. The seeds were sown at the depth of 4–5 cm and with the rate of 100 viable seeds per row (1.5 m length), interrow distance was 15 cm, 30 cm between the samples, 2 rows for sample with control row of initial variety samples after every 20 variants.

Field experiment was conducted at the Research-Education Center of Dnipro State Agrarian and Economic University (48°51'10" N 35°25'31" E). Important agricultural practices such as fertilization were provided. Assessment was conducted during 2018–2022 years.

The data were statistically analyzed using ANOVA, grouping and estimation of data was provided by discriminant and cluster analyses (Euclidian distance, single linkage) (Statistic 10.0, multivariate module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between the samples were assessed using the Tukey HSD test.

Results

The study of the obtained plant material began with identification of the overall frequency of changes in the second generation of the resulted mutant population. Also, the obtained forms were subsequently subcultivated to confirm their inheritance. In total, as control and material that had been subject to the mutagen action, we examined 13,600 families of second-third generation. The mutagen was used in the concentrations commonly used in breeding process. The number of families varied 500 to 300 per variant. The exception was the extreme concentration (DMS 0.05%). For some more susceptible varieties such as Balaton, Nyva Odeska, the sample comprised up to 200 families (Table 1).

The varieties were subdivided into two groups and data about the general mutation rate for each variety and variant are presented in

Tables 1 and 2. More susceptible varieties (as will be later shown by grouping using cluster analysis) are in Table 1 and significantly less variable in Table 2.

Table 1
General rate of mutation cases and families of second – third generations: first group (more sensitive to mutagen action) ($x \pm SD$, $n = 200–500$)

Variety	Number of selecting families	Number of mutant families	Rate of mutations, %
Balaton	500	2	0.40 ± 0.10 ^a
Balaton, DMS 0.0125%	500	52	10.40 ± 0.59 ^b
Balaton, DMS 0.025%	300	67	22.33 ± 0.92 ^c
Balaton, DMS 0.050%	200	43	21.50 ± 0.88 ^c
Zoloto Ukrainy	500	6	1.20 ± 0.24 ^a
Zoloto Ukrainy, DMS 0.0125%	500	46	9.20 ± 0.67 ^b
Zoloto Ukrainy, DMS 0.025%	400	78	19.50 ± 0.89 ^c
Zoloto Ukrainy, DMS 0.050%	350	64	18.29 ± 0.84 ^c
Zelenyi Hai	500	3	0.60 ± 0.20 ^a
Zelenyi Hai, DMS 0.0125%	500	51	10.20 ± 0.65 ^b
Zelenyi Hai, DMS 0.025%	400	71	17.75 ± 0.78 ^c
Zelenyi Hai, DMS 0.050%	300	62	20.67 ± 0.91 ^d
Nyva Odeska	500	3	0.60 ± 0.21 ^a
Nyva Odeska, DMS 0.0125%	500	51	10.20 ± 0.54 ^b
Nyva Odeska, DMS 0.025%	300	60	20.00 ± 0.89 ^c
Nyva Odeska, DMS 0.050%	200	35	17.50 ± 0.76 ^d

Note: indicate significant differences at $P < 0.05$ according to the Tukey HSD test with the Bonferroni Correction; comparison in terms of one variety in line.

As can be seen (in addition), the more variable varieties were also among those that were more subjected to depressive effects on growth and development in the first generation. There were varieties Balaton (general rate up to 22.3%), Zoloto Ukrainy (up to 19.6%), Zelenyi Hai (up to 20.7%), Nyva Odeska (up to 20.0%). In pairwise comparison, we found no significant difference between the concentrations of DMS 0.025% and DMS 0.050% for Balaton and Zoloto Ukrainy ($F = 4.02$; $F_{0.05} = 5.11$; $P = 0.07$), i.e. with a certain drop in frequency, we observed a plateau between the concentrations. For the other two varieties, there were differences in the dynamics of change. The Zelenyi Hai variety demonstrated a gradual increase in frequency with increasing concentration, while the frequency of the Nyva Odeska variety decreased significantly. Therefore, concentrations close to DMS 0.025% could be considered optimal in most cases (except for the Zelenyi Hai variety). For most of the genotypes, the critical value of the mutagen in terms of induced biodiversity ranged DMS 0.025% – DMS 0.050%.

Factor analysis revealed a statistically significant difference from the varieties of the second group ($F = 14.22$; $F_{0.05} = 5.16$; $P = 0.003$). Subject to the highest concentration, the parameters varied 19.6% to 22.3%. Table 2 shows the general rate of mutations after subject to the same concentration for the genotypes of the second group in the varieties Borovytsia (18.6%), Kalancha (25.0%), Polianka (17.8%) Pochaina (18.3%), i.e. at the level of 17–18%. This was significantly lower than in the varieties of the first group, and also the variability within the group was much lower, except for variety Kalancha with very high rate for DMS 0.05%. It is these exceptions that may in the future become the basis for the optimal genotype-mutagenic combination in terms of the emergence of new valuable forms. However, one should consider the number and magnitude of altered traits in the spectrum of mutations at the next stage of this study. When comparing in pairs, we found that all varieties were characterized by a gradual significant increase in frequency with increasing concentration. The group was stable and uniform in this trait, the highest values seen for DMS 0.05%.

However, the mutagen action was statistically significant for both groups, both for the variance in the change in mutagen concentration (for first group $F = 156.11$; $F_{0.05} = 3.86$; $P = 3.02 \cdot 10^{-10}$; for second group $F = 124.09$; $F_{0.05} = 3.86$; $P = 1.17 \cdot 10^{-9}$) and for individual genotypes (for first group $F = 17.47$; $F_{0.05} = 4.11$; $P = 0.01$; for second group $F = 29.36$; $F_{0.05} = 4.11$; $P = 0.001$).

In general, all varieties belong to stable genotypes and the level of spontaneous variability was low. Moreover, as for modern varieties, which have significantly lower genome stability, it is even low.

Table 2

General rate of mutation cases and families in the second – third generations: second group (less sensitive to mutagen action) ($x \pm SD$, $n = 300-500$)

Variety	Number of selected families	Number of mutant families	Rate of mutations, %
Borovytsia	500	4	0.80 ± 0.11 ^a
Borovytsia, DMS 0.0125%	500	48	9.60 ± 0.39 ^b
Borovytsia, DMS 0.025%	400	67	16.75 ± 0.68 ^c
Borovytsia, DMS 0.050%	350	65	18.57 ± 0.83 ^d
Kalancha	500	5	1.00 ± 0.22 ^a
Kalancha, DMS 0.0125%	500	47	9.40 ± 0.58 ^b
Kalancha, DMS 0.025%	400	85	21.25 ± 0.89 ^c
Kalancha, DMS 0.050%	300	75	25.00 ± 1.09 ^d
Polianka	500	2	0.40 ± 0.20 ^a
Polianka, DMS 0.0125%	500	40	8.00 ± 0.33 ^b
Polianka, DMS 0.025%	450	57	12.67 ± 0.45 ^c
Polianka, DMS 0.050%	400	71	17.75 ± 0.69 ^d
Pochaina	500	2	0.40 ± 0.19 ^a
Pochaina, DMS 0.0125%	500	42	8.40 ± 0.41 ^b
Pochaina, DMS 0.025%	450	59	13.11 ± 0.50 ^c
Pochaina, DMS 0.050%	400	73	18.25 ± 0.72 ^d

Note: see Table 1.

The cluster analysis carried out according to the total mutation frequency showed (Fig. 1) that, in general, the varieties could be distinctly divided into four groups according to a genotype-mutagenic interaction. The first group comprises the more DMS-susceptible varieties: Borovytsia, Zelenyi Hai, Nyva Odeska, and Zoloto Ukrainy. The second main group includes varieties Polianka, Pochaina, which had a somewhat less variability after subject to the action of all concentrations. Varieties Kalancha and Balaton were each distinguished into two individual minor groups respectively (regarding special action of DMS 0.025% concentration). Apparently, this was due to the dynamics of the change in general rate depending on the concentration of the mutagen; there can be no one other reasons.

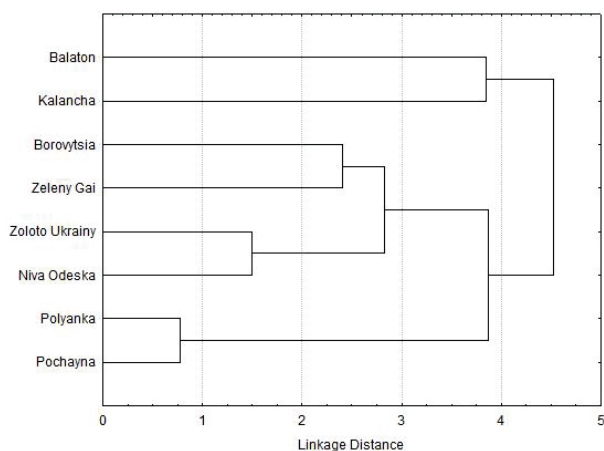


Fig. 1. Results of cluster analysis by general mutation rate, single linkage, Euclidean distance

More interesting was the study of the complex indicator of the level of changeability, which takes into account not only the number of mutation cases under the mutagenic factor action, but also the number of changed traits (the spectrum of changes, i.e., the number of traits that underwent changes under the action of a certain mutagen concentration) (Table 3 for the first group of varieties and Table 4 for the second group, the cluster analysis data for this parameter are presented in Figure 2).

Based on the presented data on the level of changeability, we found that there were also statistically significant changes with each concentration ($F = 112.26$; $F_{0.05} = 3.86$; $P = 5.17 \cdot 10^{-8}$) and depending on the genotype of the initial material ($F = 17.78$; $F_{0.05} = 4.11$; $P = 0.002$), and also differences between the two groups of varieties ($F = 12.34$; $F_{0.05} = 5.16$; $P = 0.01$).

At the same time, for the first group, the level of variability at the concentration (DMS 0.025%) ranged (variety Balaton) to 6.48–6.82

(variety Zelenyi Hai, subject to DMS 0.050%). Nonetheless, for three cultivars, a sharp drop in variability was observed at DMS 0.050%, and in all cases the difference between cultivars was statistically significant. Although this parameter in the variety increased, it was not that significant and the number of changed traits has even slightly decreased. Therefore, taking into account the complexity of changes demonstrated below, it is more rational to use the DMS concentration of 0.025% for the first group. At the same time, there was no significant plateau in the variability depending on concentration.

Table 3

Level of changeability caused by mutation variability: first group (more sensitive to mutagen action) ($x \pm SD$, $n = 200-500$)

Variety	Changed traits	Level of changeability
Balaton	2	0.01 ± 0.01 ^a
Balaton, DMS 0.0125%	23	2.39 ± 0.17 ^b
Balaton, DMS 0.025%	29	6.48 ± 0.26 ^c
Balaton, DMS 0.050%	25	5.38 ± 0.18 ^d
Zoloto Ukrainy	6	0.07 ± 0.02 ^a
Zoloto Ukrainy, DMS 0.0125%	22	2.02 ± 0.11 ^b
Zoloto Ukrainy, DMS 0.025%	32	6.24 ± 0.24 ^c
Zoloto Ukrainy, DMS 0.050%	26	4.75 ± 0.17 ^d
Zelenyi Hai	3	0.02 ± 0.01 ^a
Zelenyi Hai, DMS 0.0125%	27	2.75 ± 0.15 ^b
Zelenyi Hai, DMS 0.025%	34	6.04 ± 0.21 ^c
Zelenyi Hai, DMS 0.050%	33	6.82 ± 0.23 ^d
Nyva Odeska	3	0.02 ± 0.01 ^a
Nyva Odeska, DMS 0.0125%	22	2.24 ± 0.14 ^b
Nyva Odeska, DMS 0.025%	30	6.00 ± 0.23 ^c
Nyva Odeska, DMS 0.050%	22	3.85 ± 0.16 ^d

Note: see Table 1.

Table 4

Level of changeability, caused by mutation variability: second group (less sensitive to mutagen action) ($x \pm SD$, $n = 300-500$)

Variety	Changed traits	Level of changeability
Borovytsia	4	0.03 ± 0.01 ^a
Borovytsia, DMS 0.0125%	22	2.11 ± 0.11 ^b
Borovytsia, DMS 0.025%	32	5.36 ± 0.21 ^c
Borovytsia, DMS 0.050%	30	5.57 ± 0.22 ^c
Kalancha	5	0.05 ± 0.02 ^a
Kalancha, DMS 0.0125%	27	2.54 ± 0.18 ^b
Kalancha, DMS 0.025%	30	6.38 ± 0.23 ^c
Kalancha, DMS 0.050%	29	7.25 ± 0.25 ^d
Polianka	2	0.01 ± 0.01 ^a
Polianka, DMS 0.0125%	21	1.68 ± 0.09 ^b
Polianka, DMS 0.025%	25	3.17 ± 0.15 ^c
Polianka, DMS 0.050%	28	4.97 ± 0.20 ^d
Pochaina	2	0.01 ± 0.01 ^a
Pochaina, DMS 0.0125%	24	2.02 ± 0.11 ^b
Pochaina, DMS 0.025%	28	3.67 ± 0.19 ^c
Pochaina, DMS 0.050%	26	4.75 ± 0.22 ^d

Note: see Table 1.

For the second (more resistant to the action) group, the level of variability was 4.75 (variety Pochaina) to 7.25 (variety Kalancha). In this case, the second group was more variable and, for all the varieties, except for Borovytsia ($F = 3.11$; $F_{0.05} = 5.11$; $P = 0.09$), the level of variability increased with increasing concentration, having decrease in the number of altered traits (insignificant), except for Polianka. According to the demonstrated complexity of changes, DMS would be best to use for most of the varieties in 0.025% concentration. Zelenyi Hai and Kalancha seem to be the most promising for finding the optimal mutagen composition and initial form.

The variability within the second group was significantly lower, but the differences between the groups are very significant even during the initial assessment. Cluster analysis in this case showed a clearer division into three groups (Fig. 1) without presence of any secondary variants, while the factor space of the analysis was significantly smaller than in the first case. Thus, the evaluation according to the variability level proved to be more accurate mathematically than such according to mutation frequencies. The first group consisted of varieties Balaton, Zoloto Ukrainy, Borovytsia, Nyva Odeska, characterized by the

optimal value of variability at DMS 0.025%; the second group was represented by the Zelenyi Hai and Kalancha genotypes, which were most responsive to the action of this mutagen; the third group comprised Polianka and Pochaina, which were minimally responsive.

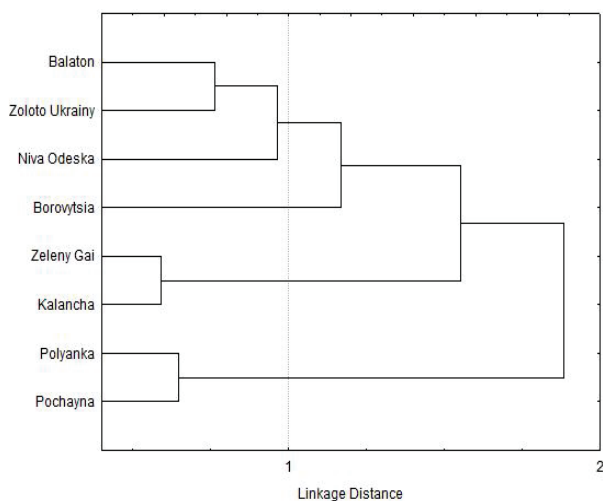


Fig. 2. Results of cluster analysis by level of variability: single linkage, Euclidean distance

In the spectrum of changes, the traits were divided into 6 groups in accordance with the generally accepted classification. A classification analysis was also carried out both for individual characteristics of the mutation process (Tables 5 and 6) and for genotypes (varieties).

The first group contains mutations in the plant architecture. These include the following signs: thick stem, thin stem, tall stem, short stalk, semidwarf, dwarf, intensive waxy coating, weak waxy coating, and presence of a waxy coating. On the whole, in the group, the most probable forms of the DMS action are high stem, short stem, semidwarfs and dwarfs (at high concentrations), and forms with a weak waxy coating and absence of waxy coating. Less pronounced mutations have been more common, with the exception of semi-dwarfs (but the latter is due to the fact that the initial material was predominantly short). The highest (up to 2.3%) probability was such of the appearance of high forms, which were present in almost every variant.

The second group included changes in wheat grain. Such traits as barrel-shaped grain, coarse grain, fine grain were observed. Only the large-grain mutation occurred more or less often (and its probability increased slightly with increasing concentration in some varieties), and the other mutations were rare. The group was difficult to identify and had little weight in the factor space.

The third group includes changes in the spike structure (the most numerous, 15 different traits). These changes tended to occur more frequently as the concentration increased. Some varieties were characterized by presence of a greater number of such changes as anthocyanin awns and a double spike. Changes in the spike shape were also common. The mutation of the spike from awn spike to awnless form (almost three times) was more frequent than from awnless spike to awn-bearing spike. Transitional forms (semi-awn spike) occurred at the level of the second variant.

The fourth group (changes in the physiology of growth and development) was the most variable, having 4 in total: sterility, early-maturing, late-maturing, and disease tolerance. More frequent (for all variants) were sterile forms, and then early ripeness and disease tolerance. At the same time, in some variants, increase in concentration led to late ripeness. Sterility was more typical for high concentrations. In general, all traits for this group were present in the model.

The fifth group includes systemic mutations leading to extremely significant changes in the spike structure, altering the cultivated form to the phenotype of its wild relatives. Such changes were most significant under the action of high concentrations of the mutagen but not only for squareheads and speltoid-spike wheat. More likely was the appearance of squareheads, which could form even at low DMS 0.0125% concentration.

The sixth group consists of valuable forms, having high grain productivity and tillering. It occurred in most varieties, except for some at the concentration of DMS 0.05%.

Table 5
Results of discriminant analyze

Variables at model	Wilks	Partial	F-remove,	P
	Lambda λ	Lambda	$F_{0.05} = 4.02$	
Mutation rate	0.10	0.82	19.44	0.01
Level of variability	0.06	0.93	20.67	0.01
First group	0.11	0.77	14.55	0.01
Second group	0.61	0.34	2.21	0.13
Third group	0.13	0.58	13.12	0.01
Fourth group	0.10	0.79	18.67	0.01
Fifth group	0.17	0.69	8.33	0.03
Sixth group	0.21	0.79	6.03	0.04

Discriminant analysis revealed model of individual parameters by groups (Table 5). The model consisted of the rate, the level of variability, mutations for the first, third, fourth (only by concentrations), fifth, and sixth groups.

Thus, for this mutagen on this material, one can confidently predict how to obtain sources for selection breeding for early ripeness, disease tolerance, plant height, but it is difficult to find valuable forms in complex, without negative changes. As for the significance realization of a trait depending on the genotype and concentration of the mutagen (Table 6), mutagen concentration is still of great importance, and genotype had no effect only in two groups.

Table 6
Factor loadings (varimax raw) for all parameters of variability

Parameter	Genotype	Concentration
Mutation rate	0.797*	0.963*
Level of variability	0.843*	0.972*
First group	0.762*	0.876*
Second group	0.242	0.313
Third group	-0.796*	-0.778*
Fourth group	0.624*	0.816*
Fifth group	0.480	0.971*
Sixth group	0.611*	-0.588*
Expl. Var	3.215	3.790
Prp. Totl	0.299	0.129

Note: * – statistically significance.

It is important that both the genotype of the initial material and the concentration of the mutagen affected the mutation rate, the level of variability, and the frequencies for the first and third-fifth groups of mutations, which somewhat contradicts the results of discriminant analysis, supplementing it with new significant parameters (the third mutation group).

The classification by varieties in the factor space showed that all genotypes can be reliably identified according to the measured parameters, varieties Kalancha and Zelenyi Hai to the lower degree. In general, all the varieties were classified successfully.

Discussion

The results make it possible to evaluate the capabilities of chemical mutagenesis and the use of highly active substances to induce genetic changes (Mangi et al., 2021; Hassine et al., 2022) or making adjustments to the initial material (variety or line), which generally meet modern requirements but need to be improved for some parameters (Abaza et al., 2020).

This mutagen is quite actively used in practice to produce new genetically- and selective-breeding-valuable forms (OlaOlorun et al., 2021), primarily due to the high variability of changes in plant structure (plant architecture) (OlaOlorun et al., 2020; le Roux et al., 2021), and the formation of plants that better meet to modern requirements to intensive varieties (Ram et al., 2019) by transforming local semi-intensive but better adapted forms (under our conditions, local varieties of national selective breeding) (le Roux et al., 2021).

On the other hand, on this material, the examined mutagen showed a slightly different range of changes than in the case of foreign scientific programs (Bezie et al., 2020; OlaOlorun et al., 2021). While the general mutation rate was significantly higher, primarily for the group of genotypes identified as less resistant to the factor of this nature, the proportion of beneficial changes was less significant than expected (Hassine et al., 2022; Nazarenko et al., 2022). Perhaps, more

thorough studies of grain quality and various types of tolerance to unfavorable environmental factors for mutant lines of older generations will significantly correct the results (Chaudhary et al., 2019; Hong et al., 2022).

It should also be noted that there was a stronger relationship between the rate and frequency of cytogenetical aberrations obtained for these varieties and the general rate and spectrum of visually identified changes. This fact has been determined in the past investigations, but not for every mutagen (Yali & Mitiku, 2022).

The use of integrative indicators, which, in addition to characterizing the total number of mutant cases, also include the the spectrum of change by the number of individual traits affected by the genetic activity of the factor, was previously noted by us as more promising (Ram et al., 2019; Nazarenko et al., 2022; Shabani et al., 2022). This was confirmed primarily for this mutagen, even with greater justification through mathematical and statistical analysis than previously for gamma-rays (Nazarenko et al., 2019), nitrosoalkylureas, or other mutagenic factors (Nazarenko et al., 2022).

As a mutagenic factor, DMS shows an extremely significant dependence on the characteristics of the genotype-mutagenic interaction, i.e. depends on the characteristics of the initial genotype material with a clear division according to genetically determined reactivity to the action of this mutagenic factor (Beiko & Nazarenko, 2022).

When monitoring all types of effects of mutagenic action, from the cellular to the general plant level, there was seen clear relationship in terms of severity of impact of this factor, which was not always the case. This indicates the nature of a rather direct (through a direct effect on the DNA structure) rather than an indirect effect on the hereditary apparatus of a plant cell (Ahumada-Flores et al., 2020).

The most important is comprehensive assessment of the general rate and spectrum of the obtained changes, which shows the success of this mutagen in inducing mutations (Spencer-Lopes et al., 2018). Consideration of only one component is meaningless and may lead to the loss of part of the data for classification analysis (OlaOlorun et al., 2020).

The examine mutagen is more valuable in terms of inducing a wide range of different changes than in terms of producing economically valuable forms and lines, at least at the level of visual identification of mutations (Ahumada-Flores et al., 2021; Von Well et al., 2022). This allows us to consider it more promising for clarifying the data on the control mechanisms of certain traits and obtaining a number of forms (Gorbatova et al., 2020) that can be used in the future not directly as commercial varieties (Gupta et al., 2019) but as a source of some valuable key traits for the partial improvement of existing varieties and lines (Bezie et al., 2020; Von Well et al., 2023).

Thus, in at least two cases, we can expect a similar effect in obtaining a higher frequency of certain valuable forms, as has been noted for a number of varieties before. The picture would more complete by detecting microchanges that could somewhat change the already obtained data and supplement them (Hase et al., 2020). However, as practice shows, this study cannot drastically change the trend in the overall frequency and spectrum of the forms obtained, that is, the results obtained cannot be significantly adjusted (Kartseva et al., 2023). First of all, additional valuable qualities of the produced lines will be identified and, secondly, data on the relationship between changes recorded and inherited at the phenotype level and biochemical and physiological changes will be expanded. This will improve the predictability of identifying new valuable lines.

Conclusion

In the studied source material, we found two genotypes that may be more promising as future components of the composition with an increased yield of altered forms under the action of the studied mutagen. In general, the site-specificity of even an agent with a high damaging ability is quite sufficient to identify the most responsive varieties. In most cases, it is still more optimal to use a concentration in a more moderate range. Quite often, for most of the source material, an increase in concentration does not lead to positive shifts and may even turn out to be negative in terms of the obtained material, both quantitatively and qualitatively. The use of this agent is promising from the point of view of obtaining valuable forms, especially undersized, with a large ear, early ripening, resistant to diseases, and productive.

However, the negative aspect is high probability of a complex of these changes with high plant growth, changes in the ear, including systemic, a high level of sterility, especially due to the high probability of complex changes at DMS 0.025–0.050%, a concentration that is reasonable to use. Thus, the use of this mutagen from a practical point of view is somewhat complicated. It is planned to conduct a study of the selected promising material, focusing on tolerance to adverse environmental factors (winter period, drought resistance, hot resistance), assess the technological qualities of grain (protein content, low- and high-molecular glutenins, gliadins), show possible changes in the content of valuable microelements in wheat grains.

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